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ORIGINAL ARTICLE

Reproductive hormones during pubertal transition in girls with transient Thelarche

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Abstract

Context: Transient thelarche (TT), that is, the appearance, regression and subsequent reappearance of breast buds, is a frequent phenomenon, but little is known about pubertal transition in these girls.

Objective: To describe pubertal progression, growth, genotypes, reproductive hormones and growth factors in girls with TT compared to those who do not present TT (non-TT).

Design: Retrospective analysis of a longitudinal population-based study.

Patients or Other Participants: Girls (n = 508) of the Chilean Growth and Obesity cohort.

Measurements: Pubertal progression, reproductive hormones, follicle stimulating hormone (FSH) beta subunit/FSH receptor gene single nucleotide polymorphisms and growth.

Results: Thirty-seven girls (7.3%) were presented TT. These girls entered puberty by pubarche more frequently (51%) than girls with normal progression (non-TT; n = 471; 23%, $P = .005$). Girls with TT who were under 8 years old had lower androgens, anti-Müllerian hormone (AMH), luteinizing hormone (LH) and oestradiol (all $P < .05$) than older girls with TT. At the time of Tanner breast stage 2 (B2), girls with TT had higher androgens, LH, FSH, IGF1, LH, insulin and oestradiol ($P < .01$) than at the time of TT. TT girls were older at B2 (10.3 ± 1.1 vs. 9.2 ± 1.2 years, $P < .001$) and menarche (12.3 ± 0.8 vs. 12.0 ± 1.0 years, $P = .040$) than their counterparts (non-TT). No differences in anthropometric variables or *FSHB/FSHR* genotypes were detected.

Conclusion: Transient thelarche is a frequent phenomenon that does not appear to be mediated by hypothalamic-pituitary-gonadal axis activation or by adiposity. Hormonal differences between earlier TT and later TT suggest that their mechanisms are different.

KEYWORDS

androgens, environmental disruptors, puberty, transient thelarche

1 | INTRODUCTION

Puberty is a process characterized by the acquisition of individual reproductive capacity and begins with the reactivation of the hypothalamic-pituitary-gonadal (HPG) axis. Re-emerging pulsatile secretion of gonadotrophin releasing hormone (GnRH) stimulates the synthesis and release of the gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, act on the gonads, activating the steroidogenic machinery and oocyte maturation.[1] According to studies carried out in families and twins, genetic factors explain more than half of the phenotypic variation in pubertal onset time.[2-4] Worldwide, over the last 15 to 20 years, the mean age at pubertal onset in girls has declined by approximately 1 year, with minimal changes in age at menarche.[5-9] The same trend has been reported in national cross-sectional and longitudinal studies during a similar period of time.[10,11] This change has been attributed at least in part to an increase in obesity rates, although results on the topic are contradictory.[7,12] It has been postulated that earlier thelarche may not always be linked to the activation of the HPG axis.[7,13,14] However, given the cross-sectional nature of most of the previous studies, the question has not been addressed in further detail.

Transient thelarche (TT) consists of the appearance, regression and subsequent reappearance of breast buds. This phenomenon has been addressed in a single longitudinal study in Caucasian children,[15] which found that its presence was associated with pubertal onset by pubarche more frequently than in controls but did not affect subsequent pubertal progression. However, the aforementioned study was limited in that it included only 12 cases of TT among 98 girls.

The Growth and Obesity Chilean Cohort Study (GOCS) is a longitudinal study designed to evaluate the impact of early nutrition on growth and development in a representative sample of Chilean girls in medium-to-low socioeconomic strata. The purpose of the present study was to evaluate whether girls with TT show differences in pubertal timing; reproductive hormones; growth factors; selected genetic polymorphisms; and other factors, such as anthropometry, that are known to be associated with pubertal timing.

2 | SUBJECTS AND METHODS

This study was performed within the GOCS cohort, which has been described elsewhere.[14] Briefly, all 3- to 4-year-old children from the southeast area of Santiago who participated in the Chilean National Nursery School Council Program during 2006 were screened for participation. All included children were born at term from singleton gestations, with birthweight ≥2500 and ≤4500 grams and no evidence of disease at birth or at the time of recruitment. Approximately 85% of recruited children agreed to participate in the study (n = 1195). There were no significant differences in age, sex, anthropometry at birth or anthropometry at enrolment time between participants and nonparticipants. Since 2009, at an average age of 6.7 years, 87% (n = 1044, 50% girls) of the original cohort was evaluated, and subsequent evaluations were performed every 6 months to record the appearance of secondary sex characteristics (Figure 1).

A total of 508 girls were included in the current analysis and were divided into two comparison groups: girls who presented TT and those who did not present TT. An intragroup comparison of the girls who presented TT was also made based on the interval between the first TT and when the thelarche became progressive (Tanner breast stage 2, B2). The information was extracted from the databases of the GOCS cohort.

The protocol was approved by the Institutional Review Board (IRB) of the Institute of Nutrition and Food Technology (INTA) at the University of Chile, and informed consent was obtained from all parents and/or guardians of the girls.

2.1 | Pubertal development evaluation

Pubertal development was assessed (at the time of each visit) and categorized by a single dietitian trained by a paediatric endocrinologist (VM) according to the Marshall and Tanner stages,[16] with constant supervision. Pubertal onset was defined as the first secondary sex characteristic detected in the examination (Tanner pubic hair stage 2 (PH2) or later) and/or thelarche (B2 or later). Pubic hair was evaluated by inspection, and breast development was evaluated by palpation. The concordance (kappa) for the pubertal evaluation between the

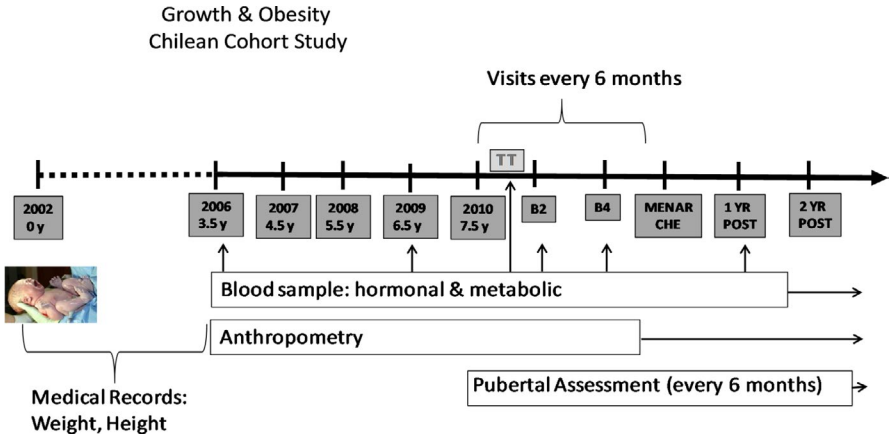

FIGURE 1 Diagram of the Growth and Obesity Chilean Cohort Study (GOCS) with years and chronological ages indicating time from birth (year 2002)

nutritionist and VM was 0.9.[17] If thelarche or pubarche occurred between two visits, the onset was defined as the midpoint between these dates. The larger of the two breasts determined a girl's breast stage.

2.2 | Transient thelarche

Transient thelarche was defined as the appearance and subsequent regression of the breast bud between visits. For the girls with TT, the first breast development was not regarded as pubertal onset; pubertal onset was defined as onset of progressive (at an advanced stage) pubertal development (B2+ or PH2+). After the detection of B2+ or PH2+, follow-up was continued at the INTA facilities every 6 months until 1 year after menarche. The age of maternal menarche was obtained by self-report.

2.3 | Anthropometric measurements

Weight and height were measured annually according to standardized protocols. Height was measured using a Harpenden stadiometer with a sensitivity of 1 mm. Weight was quantified using a TANITA BC-418, which has an accuracy of 0.1 kg. Weight and height prior to recruitment (0-3 years) were obtained from health supervision records. The validity of these data had been previously verified.[17] The evaluation of age-specific body mass index (BMI) standard deviation score (SDS) was based on the references of the World Health Organization (WHO) for 2007.

2.4 | Laboratory examinations

Fasting blood samples were collected at the time of appearance of thelarche (transient (TT) and progressive (B2)), at Tanner breast stage 4 and one year after menarche (early follicular phase, between days 1 and 7 of the cycle, before 8:30 AM).

The samples were analysed at the Endocrinology Laboratory of the IDIMI. Testosterone (T), androstenedione (A) and dehydroepiandrosterone sulphate (DHEAS) were determined by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) as we have previously described.[18] Briefly, the samples were analysed by HPLC-MS/MS with an Agilent 1260 HPLC system (Santa Clara, CA, USA) coupled to an AB Sciex 3200 Quantum Ultra triple quadrupole mass spectrometer (Foster City, CA, USA). The liquid chromatography separation was carried out on a 150-mm-long column with an internal diameter of 300 μm, packed with 4 μm Synergi Hydro-RP particles and maintained at 40°C.

The samples were processed by a Chromsystems kit (Chromsystems Instruments & Chemicals, Gräfelfing, Germany). Samples, calibrators and quality controls were run in duplicate and prepared according to the manufacturer's instructions. The lower limits of detection for DHEAS, A and T were 0.204 μmol/L, 0.105 nmol/L and 0.031 nmol/L, respectively. The corresponding intra-assay coefficients of variation (CVs) were 3.8, 3.3 and 1.8%, respectively, and the corresponding interassay CVs were 8.3, 4.7 and 4.4%, respectively.

LH and FSH in serum (S = 0.06 mIU/mL) and sex hormone-binding globulin (SHBG) (S = 0.5 nmol/L) were measured by radioimmune assays (RIAs; kits were supplied by Izotop Laboratories (Hungary). The intra-assay/interassay CVs for FSH, LH and SHBG were 4.0%/5.3%, 4.5%/5.6% and 3.9%/6.9%, respectively. Serum oestradiol was measured by RIA (Pantex, Santa Mónica CA, USA). The sensitivity of this assay is 18.4 pmol/L, and its intra-assay and interassay CVs are 5.7% and 7.9%, respectively. IGF1 was measured by RIA in house prior to extraction, as previously described.[19] The sensitivity of this assay is 0.65 nmol/L, and its intra-assay and interassay CVs are 8.6% and 10.2%, respectively. Serum anti-Müllerian hormone (AMH) was quantified by ELISA (Beckman Coulter Inc, Brea, CA, USA). The sensitivity of this assay was 0.71 pmol/L, and the intra-assay and interassay CVs were less than 5%. SHBG and testosterone were used to calculate the free androgen index (FAI) as previously reported.[20]

2.5 | Genotyping

Genomic DNA was obtained from peripheral blood (0.2 mL preserved in EDTA), and single nucleotide polymorphism (SNP) genotyping was performed with KASP assays (LGC Genomics, Hoddesdon, UK) in Denmark (Department of Growth and Reproduction). The following SNPs previously associated with thelarche[21] were genotyped: *FSHB* −211G > T (rs10835638), *FSHR* −29G > A (rs1394205) and *FSHR* c.2039A > G (rs6166) in 434 girls.

2.6 | Statistical analysis

The data were collected prospectively but for the current analysis we did a retrospective analysis. Data are presented as averages, medians or proportions, according to the distributions of the variables along with their corresponding measures of dispersion. Based on those criteria, the girls' ages at the time of each of the pubertal milestones, as well as BMI SDS and height SDS, were compared between participants with TT and non-TT for all evaluation times by Student's t test or the Mann-Whitney *U* test for quantitative variables and the chi-squared test of independence for qualitative variables. In the group of girls who presented TT episodes, an intragroup comparison of hormonal profiles between the time of TT and the time of progressive thelarche (B2) was performed using Student's t test for paired samples or the Wilcoxon signed rank test for related samples, depending on the distribution of the variables. In addition, an intragroup hormonal evaluation was performed in girls with TT according to whether TT emerged before 8 years or a ≥8 years based on the normal definition of age at thelarche (older than 8 years).[22] The hormonal variables were evaluated according to age and BMI to correct for nutritional status (normal, overweight or obese).

A linear regression model of the hormonal concentration profile (DHEAS, testosterone, androstenedione, IGF1, AMH and LH) in girls with TT and girls without TT was developed at B4 and 1 year after menarche. The regression is shown for a crude model and a model adjusted by chronological age and BMI SDS at sampling time.

Fisher's exact test was used to compare the frequency of pubertal onset by pubarche, thelarche or a combination of pubarche and thelarche between the group of girls with TT and the rest of the girls from the longitudinal cohort. The same test was applied to determine whether the distribution of minor *FSHB*/*FSHR* alleles differed between girls with and without TT and/or between girls with TT and a general sample of Caucasian females from 1000 Genomes. The database was stored in REDCap Software version 6.11.1 (© 2018 Vanderbilt University) and processed with STATA (version 14, Stata Corp, College Station, TX).

3 | RESULTS

3.1 | Pubertal development in girls with TT

Thirty-seven girls of the 508 girls (7.3%) in the study population were presented TT, either bilaterally or unilaterally. All of the girls were in Tanner stage 2 except for 2 who were in Tanner stage 3. The mean age at presentation of TT was 7.9 ± 1.4 years, with 65% (n = 24) presenting TT before the age of 8 years. Among the non-TT girls (n = 471), 19.1% (n = 91) developed premature thelarche, that is, before the age of 8 years (Table 1). Pubertal development started with pubarche more frequently in girls with TT than in those without TT (51% versus 23%; $P < .05$), and the subjects with TT reached B2 and menarche at significantly later ages than the subjects without TT. In addition, the intervals from B2 to B4 ($P < .05$) and from B2 to menarche ($P < .005$) were shorter in the girls with TT than in the girls without TT (Table 1).

Among girls who presented TT before the age of 8 years, 33% started puberty by pubarche, compared to 85% of girls who presented TT after the age of 8 years. *The mean age at TT in girls who presented TT before 8 years old was 7.7 ± 0.7, compared to 9.7 ± 0.6 years in girls with TT after 8 years old*. The mean age at B2 in girls who presented TT before 8 years old was 9.8 ± 0.9 years, compared to 11.2 ± 0.7 years in girls who presented TT after 8 years old ($P < .001$). The time interval between B2 and B4 was 1.5 ± 0.8 years in the former group, compared to 0.9 ± 0.2 years (p = ns) in the latter group. Furthermore, the time interval between TT and menarche was 4.5 ± 1.0 years in the former group versus 2.8 ± 0.7 years ($P < .001$) in the latter group.

3.2 | Pattern of reproductive hormones and growth factors

3.2.1 | Intragroup hormone concentrations in girls with TT between the times of TT and B2

In girls with TT, the hormone concentrations at transient vs progressive thelarche (B2) differed significantly. We observed significantly lower concentrations of DHEAS, T, A, IGF1, AMH, LH, insulin and oestradiol at TT than at progressive thelarche (B2). Moreover, the difference remained significant when adjusted for BMI SDS and age at sampling. Furthermore, FSH, SHBG and calculated FAI also became significantly different after adjustment (Table 2).

3.2.2 | Intragroup hormone concentrations in girls with TT according to whether TT emerged before 8 years or at ≥8 years

Girls who presented TT before 8 years had lower concentrations of T, A and LH and a lower FAI than those who presented TT at 8 years or older (Table 3). Moreover, when these concentrations were adjusted for BMI SDS and age at sampling, significant differences remained, and the concentrations of DHEAS and oestradiol were also

TABLE 1 Age at presentation (years) of pubertal milestones and velocity of progression in girls with and without TT

Age	Group with TT (n = 37) mean, SD	Group without TT (n = 471) mean, SD	*P*-value
Age at TT (years)	7.9 ± 1.4		
Girls with TT before 8 years old, n (%)	24 (65%)		
Age at B2 (years)	10.3 ± 1.1	9.2 ± 1.2	<.001
Girls with B2 before 8 years old, n (%)	none	91 (19%)	<.001
Pubarche (years)	9.4 ± 1.1	9.6 ± 1.0	.170
Pubarche before B2, n (%)	19 (51%)	108 (23%)	.005
Age at B4 (years)	11.2 ± 0.9	10.9 ± 0.9	.221
Age at menarche (years)	12.3 ± 0.8	12.0 ± 1.0	.040
Time between B2 and B4 (years)	1.37 ± 0.7	1.79 ± 0.9	.021
Time between B2 and menarche (years)	2.19 ± 0.8	2.74 ± 1.1	.005
Maternal age of menarche	12.8 ± 1.8	12.7 ± 1.7	.706

Note: Data are presented as the mean ± SD.

TABLE 2 Hormone concentrations during TT and B2 in girls with TT, first unadjusted, then adjusted by chronological age and BMI SDS at sampling time

	Unadjusted			Age + BMI SDS		
	TT	B2	*P* value*	TT	B2	*p* value*
DHEAS µmol/L	0.76 ± 0.38	1.76 ± 0.58	<.001	0.76 ± 0.17	1.77 ± 0.11	<.001
Testosterone nmol/L	0.14 ± 0.07	0.28 ± 0.17	.013	0.10 ± 0.03	0.28 ± 0.14	<.001
Androstenedione nmol/L	0.59 ± 0.24	1.08 ± 0.49	.005	0.59 ± 0.07	1.08 ± 0.28	<.001
IGF-I nmol/L	21.8 ± 4.7	30.7 ± 7.7	<.001	21.4 ± 1.2	30.7 ± 1.8	<.001
AMH pmol/L	21.4 ± 12.1	32.1 ± 16.4	.002	21.4 ± 4.3	31.4 ± 2.9	<.001
LH UI/L	0.15 ± 0.09	0.88 ± 1.17	<.001	0.15 ± 0.07	0.88 ± 0.58	<.001
FSH UI/L	2.30 ± 1.6	3.10 ± 2.60	.058	2.30 ± 0.20	3.10 ± 1.50	.013
SHBG nmol/L	65.8 ± 30.4	57.4 ± 28.9	.118	66.7 ± 22.2	57.4 ± 21.3	<.001
Insulin mUI/L	7.6 ± 2.5	13.7 ± 7.9	.001	7.7 ± 1.4	8.3 ± 1.4	<.001
Oestradiol pmol/L	22.0 ± 11.0	77.1 ± 58.7	.001	22.0 ± 2.9	80.8 ± 25.7	<.001
FAI	0.30 ± 0.30	0.80 ± 0.90	<.001	0.20 ± 0.20	0.80 ± 0.80	<.001

Note: Data are presented as the mean ± SD.
*Paired t test.

TABLE 3 Hormone concentrations in girls with TT according to the age of presentation: under 8 years (n = 24) or over 8 years (n = 13), first unadjusted, then adjusted by chronological age and BMI SDS at sampling time

	Girls with TT Unadjusted			Unadjusted		
	<8 y n = 23	≥8 y n = 11	p value	TT B2 n = 37	non-TT B2 n = 471	*P* value
DHEAS µmol/L	0.69 ± 0.39	0.91 ± 0.32	0.053	1.76 ± 0.58	1.75 ± 0.93	.493
Testosterone nmol/L	0.10 ± 0.07	0.17 ± 0.07	0.002	0.28 ± 0.17	0.24 ± 0.14	.289
Androstenedione nmol/L	0.52 ± 0.17	0.70 ± 0.28	0.051	1.08 ± 0.49	0.98 ± 0.56	.279
IGF-I nmol/L	21.7 ± 4.2	21.0 ± 3.8	0.606	30.7 ± 7.7	31.4 ± 9.1	.935
AMH pmol/L	19.3 ± 11.4	24.3 ± 13.6	0.265	28.6 ± 16.4	30.0 ± 17.1	.698
LH UI/L	0.11 ± 0.02	0.21 ± 0.12	0.003	0.88 ± 1.17	0.5 ± 0.52	.788
FSH UI/L	2.30 ± 1.8	2.40 ± 1.20	0.353	3.00 ± 2.30	2.70 ± 1.70	.770
SHBG nmol/L	70.5 ± 30.0	60.9 ± 31.9	0.397	57.4 ± 28.9	64.8 ± 25.7	.222
Insulin mUI/L	8.8 ± 3.2	9.5 ± 2.2	0.580	13.7 ± 7.9	8.8 ± 3.9	<.001
Oestradiol pmol/L	22.0 ± 7.3	25.7 ± 11.0	0.064	77.1 ± 58.7	62.4 ± 44.1	.323
FAI	0.16 ± 0.13	0.45 ± 0.40	0.015	0.80 ± 0.90	0.50 ± 0.50	.372

Note: Data are presented as the mean ± SD. Wilcoxon rank-sum test.

significantly lower in girls who presented TT before 8 years of age than in those who developed TT later (Table 3).

3.3 | Intergroup comparison of hormone concentrations in B2 in girls with and without TT

The concentrations of LH, DHEAS, T, A, IGF1, AMH, FSH, SHBG and oestradiol at the time of B2, as well as the FAI at that time, did not differ between girls who presented TT and those who did not. Only insulin concentrations were higher in girls with a history of TT than in their counterparts. When these concentrations were adjusted for BMI SDS and chronological age at blood sampling, LH, insulin and oestradiol were increased and AMH was decreased in girls with TT (Table 4).

3.4 | Intergroup comparison during B4 and 1 year after menarche in girls with and without TT

During the follow-up, no differences were found in anthropometry (BMI SDS and height SDS) between girls who presented TT and those who did not present TT (Table S1). Using linear regression analysis,

TABLE 4 Hormone concentration profile during B2 in girls with TT (n = 37) versus girls without TT (n = 471) for the unadjusted model and the model adjusted by chronological age and BMI SDS at sampling

	Age + BMI SDS			Age + BMI SDS		
	<8 y n = 23	≥ 8 y n = 11	*P* value	TT B2 n = 37	non-TT B2 n = 471	*P* value
DHEAS µmol/L	0.70 ± 0.22	0.89 ± 0.17	.019	1.77 ± 0.11	1.75 ± 0.29	.930
Testosterone nmol/L	0.10 ± 0.03	0.17 ± 0.03	<.001	0.28 ± 0.14	0.24 ± 0.07	.372
Androstenedione nmol/L	0.59 ± 0.17	0.70 ± 0.07	<.001	1.08 ± 0.28	0.98 ± 0.28	.301
IGF-I nmol/L	21.7 ± 3.7	20.7 ± 2.9	.329	30.7 ± 1.8	31.4 ± 2.9	.291
AMH pmol/L	23.6 ± 10.0	25.0 ± 8.6	.029	27.8 ± 5.0	30.0 ± 2.2	<.001
LH UI/L	0.11 ± 0.04	0.21 ± 0.09	.002	0.88 ± 0.58	0.5 ± 0.17	<.001
FSH UI/L	2.30 ± 0.50	2.50 ± 0.60	.536	3.00 ± 0.90	2.70 ± 0.60	.137
SHBG nmol/L	69.4 ± 20.0	63.9 ± 24.5	.249	57.4 ± 21.3	64.8 ± 13.7	.188
Insulin mUI/L	8.9 ± 1.1	9.5 ± 1.4	.492	13.7 ± 4.8	8.8 ± 1.0	<.001
Oestradiol pmol/L	22.0 ± 1.8	25.7 ± 3.7	.001	77.1 ± 25.7	62.4 ± 11.0	.003
FAI	0.16 ± 0.06	0.41 ± 0.29	.002	0.80 ± 0.80	0.50 ± 0.30	.415

Note: Data are presented as the mean ± SD.

we found that BMI SDS- and age-adjusted AMH levels 1 year after menarche were 6.00 pmol/L higher in girls with TT than in those without TT (Table 5).

3.5 | Other factors known to be associated with pubertal timing

We did not observe any differences between girls with TT and non-TT girls for maternal age at menarche or childhood anthropometry (BMI SDS and height SDS from birth to 7 years) (Table S1). The girls who presented transient thelarche before 8 years of age and those who presented TT after 8 years of age had no differences in the distribution of BMI at presentation of TT, with 54% versus 62% normal weight, 25% versus 8% overweight and 21% versus 31% obesity ($P = .52$), respectively. No differences were observed in height SDS (0.43 ± 0.91 in TT < 8 years versus −0.002 ± 0.82 in TT ≥ 8 years; $P = .166$). Similarly, the frequency of the investigated genetic variants in *FSHR*/*FSHB* was not associated with the development of TT (Tables S2 and S3).

4 | DISCUSSION

In this longitudinal study of healthy girls, TT occurred in 7.3% of participants. These girls entered puberty by pubarche more frequently than girls with normal progression (non-TT). Girls with TT who were younger than 8 years had lower androgens, AMH, LH and oestradiol than older girls with TT. Girls with a history of TT had higher androgens, LH, FSH, IGF1, LH, insulin and oestradiol at B2 than at the time of TT. Girls with TT were older at B2 and at menarche than their counterparts (non-TT). Girls with and without TT did not present anthropometric differences (BMI SDS or height SDS) from birth to 7 years, and their mothers' age at menarche was not different either. In addition, there was no evidence of a significant relationship between genetic variants associated with pubertal onset (the beta subunit of FSH (*FSHB*) and the FSH receptor (*FSHR*)) (22-24) and the phenomenon of TT, which is in agreement with the findings of Lindhardt Johansen et al.[15]

An increase in the sensitivity of oestrogen receptor alpha (ERα) in breast tissue has also been postulated as a trigger for thelarche, but thelarche has not yet been found to be significantly associated with the presence of variants of this receptor.[23]

Once progressive puberty developed, twice as many girls with TT as girls without TT entered the pubarche pathway. In particular, the girls who presented TT after 8 years of age began with pubarche more often than those who presented TT at a younger age. All these findings agree with the results found by Lindhardt Johansen et al.[15] However, we found that girls with TT reached B2 ~ 1 year later and menarche ~4 months later than girls without TT and had a shorter interval from B2 to menarche. These results are somewhat different from those of the abovementioned study. The prior study found that girls with TT presented definitive B2 ~ 10 months earlier than girls who did not present TT. Nevertheless, the sample in that study was composed of 12 girls, and of those girls, only one girl with TT experienced initial breast budding earlier than 8 years. We hypothesize that the delayed time of pubertal onset in our group of TT girls may be due to the inhibitory effect of peripheral hormones.

The comparison of the hormone concentration profiles in TT patients during TT and B2 showed lower concentrations of all androgens, gonadotrophins, AMH, IGF1, insulin and oestradiol at TT, suggesting that the phenomenon of TT is not triggered by activation of the HPG axis, as was previously proposed by Juul et al.[15] Nevertheless, TT could represent partial activation of the HPG axis, which cannot be detected by most commercial assays; we wonder whether GnRH analogue stimulation tests would have been a more

TABLE 5 Linear regression model of hormone concentrations in girls with TT (n = 37) vs girls without TT (n = 471)

	Linear regression: TT vs non-TT B4		Linear regression: TT vs non-TT 1 year after menarche	
	Model 1	Model 2	Model 1	Model 2
	Unadjusted Coef. (95% Conf. Interval)	Adjusted Coef. (95% Conf. Interval)	Unadjusted Coef. (95% Conf. Interval)	Adjusted Coef. (95% Conf. Interval)
DHEAS	0.30 (−19.81; 20.41)	−3.71 (−23.60; 16.18)	−14.05 (−34.30; 6.19)	−16.83 (−37.03; 3.38)
Testosterone	−0.02 (−0.05; 0.02)	−0.02 (−0.05; 0.02)	−0.01 (−0.04; 0.03)	−0.01 (−0.04; 0.03)
Androstenedione	−0.11 (−0.25; 0.03)	−0.11 (−0.25; 0.03)	−0.03 (−0.19; 0.12)	−0.06 (−0.22; 0.09)
IGF-1	−13.80 (−42.03; 14.44)	−13.09 (−41.27; 15.09)	9.60 (−13.93; 33.12)	8.60 (−14.80; 31.99)
AMH	0.45 (−0.18; 1.08)	0.52 (−0.11; 1.14)	0.91 (0.10; 1.71)	0.84 (0.03; 1.64)
LH	0.79 (−0.51; 2.09)	0.87 (−0.43; 2.18)	−0.52 (−1.84; 0.79)	−0.47 (−1.78; 0.84)
FSH	0.23 (−0.56; 1.03)	0.28 (−0.51; 1.07)	−0.51 (−1.59; 0.56)	−0.43 (−1.51; 0.65)
SHBG	−6.13 (−14.52; 2.25)	−3.73 (−11.57; 4.10)	3.97 (−3.37; 11.32)	5.26 (−1.31; 11.84)
Insulin, µUI/ml	0.60 (−2.62; 3.83)	−0.47 (−3.52; 2.59)	−2.41 (−7.03; 2.22)	−0.89 (−5.40; 3.62)
Oestradiol	−11.53 (−25.40; 2.35)	−10.89 (−24.83; 3.04)	−6.11 (−21.82; 9.60)	−5.93 (−21.85; 9.98)
FAI	0.14 (−0.39; 0.66)	−0.02 (−0.53; 0.49)	−0.19 (−0.78; 0.40)	−0.30 (−0.85; 0.26)

Note: Data are shown for the unadjusted model and the model adjusted by chronological age and BMI SDS at sampling.

Model 1: unadjusted.

Model 2: adjusted by age and BMI SDS at sampling.

sensitive way to investigate this question. Our study also adds to the information provided by the previous publication, that is, a lack of association with an increase in the peripheral conversion of androgens to oestradiol in the younger subgroup with TT, which was one of the hypotheses of the genesis of TT in the Danish study.

In girls with TT, increased concentrations of insulin at B2 were found, supporting the idea that the CNS activates the cascade that stimulates the production of gonadotrophins.[24] In a study by Sørensen et al, increased levels of insulin and insulin-like growth factor (IGF1) were observed in girls with central precocious puberty.[25] Furthermore, insulin and IGF1, both of which are increased in obesity, also directly stimulate cell division in breast tissue.[26,27]

These findings could explain the increased frequency of pubertal onset with pubarche, since insulin is a strong stimulator of ovarian and adrenal androgenic synthesis.[28] However, these differences did not persist, and only AMH concentrations remained elevated 1 year after menarche. The future risk of polycystic ovary syndrome and diabetes must be considered. The potential future health complications associated with the earlier (albeit transient) initial development of breast tissue in girls with TT, such as breast cancer and cardiovascular disease, are not well known; follow-up studies should confirm whether this TT process is completely benign.

We detected some interesting differences when analysing the girls who presented TT before 8 years of age, that is, premature thelarche. These girls had lower androgens, AMH, LH and oestradiol than girls who presented TT at 8 years or later, despite showing no differences in BMI SDS or height SDS. Furthermore, no differences in the concentrations of insulin, IGF1 or 'maturation hormones' were detected.[25,29-31] We performed this age-dependent analysis because it is well known that girls below the age of eight years have lower sex steroid concentrations than older girls, independent of thelarche or any other signs of pubertal progression.[32,33] These findings suggest differences in the aetiology of TT between the two groups. We hypothesize that girls who presented TT at or above 8 years of age may have experienced peripheral aromatization of the androgens causing

TT, whereas girls who underwent this event earlier had environmental exposures more than an endogenous production of oestradiol. In a previous study of this cohort,[34] girls who presented thelarche before the age of 8 years had elevated levels of oestradiol as measured by an ultrasensitive technique; this hormonal difference was not associated with adiposity, insulin, leptin, IGF1, DHEAS or maternal age at menarche. Other previous reports also did not show a direct association between BMI and oestradiol[35,36]; thus, these oestradiol concentrations could reflect endocrine disruptors acting as 'oestradiol-like' substances and appearing as oestradiol on the ultrasensitive test used. In addition, in a previous study of girls from this cohort, those girls who presented DHEAS levels >42.0 μg/dL at 6.8 years, which is compatible with biochemical premature adrenarche, had a 2.6 times the risk of premature thelarche and 3 times the probability of pubarche compared to girls without this phenomenon.[14] Our present results in girls with TT below 8 years support the notion that this is not a phenomenon mediated by increased peripheral aromatization of DHEAS.

In a study conducted by Biro et al,[32] increased concentrations of DHEAS were observed from 18 to 30 months, A and oestrone from 12 to 18 months and oestradiol and T from 6 to 12 months before pubertal onset. These changes were accompanied by a decrease in SHBG during those periods. The authors postulated that oestrogen production from peripheral aromatization of these adrenal androgens was the trigger for the activation of the HPG axis.[32] These hormonal findings are consistent with our results; girls at B2 in their pubertal development had higher concentrations of adrenal and ovarian androgens than they had had at TT. However, the interpretation that these concentrations trigger the activation of the HPG axis remains a hypothesis, since there is only a temporal association, and girls with TT started B2 later than those without TT.

This study is not without limitations. There is a possibility that cases of TT could have been missed, since girls were examined only once every 6 months. Second, we did not evaluate environmental endocrine disruptors.[37-40] Exposure to certain disruptors in the prenatal period has been linked to the time of onset of pubertal development.[41] Most environmental endocrine disruptors act as oestrogen and androgen agonists or antagonists, ultimately leading to a central (neuroendocrine) or peripheral disruption of the HPG axis. Humans are exposed to a mixture of multiple environmental endocrine disruptors at the same time. In a previous cohort study, we found that some compounds were associated with an early onset of menarche (by 7 months), while others were associated with an approximately 5-month delay of both thelarche and menarche.[42] Elevated serum concentrations of polybrominated diphenyl ethers (PBDEs) have been reported in girls with premature thelarche, which could be the underlying phenomenon in some girls with TT but not in girls with precocious puberty. Recent evidence indicates that both boys and girls may experience alterations in pubertal timing, with early emergence of some of the secondary sex characteristics but delayed completion of puberty. Results concerning endocrine disruptors should be interpreted cautiously, since these compounds require sustained exposure to be detected. The influence of endocrine disruptors was beyond the scope of this study. Nevertheless, the study also has important strengths; for example, it includes the largest-ever sample of reported TT cases, all of which were detected in long-term follow-up of a group of girls until they completed puberty. Second, the pubertal assessment is based on a single dietitian with permanent supervision of a single paediatric endocrinologist (V.M). Another important aspect validating our results pertains to the complete anthropometric evaluations and hormonal and genetic studies conducted throughout the follow-up, which allowed us to analyse the predictive factors and elements of pubertal progression associated with TT.

In conclusion, we showed that TT is a frequent event associated with mild changes in reproductive hormones. The long-term follow-up of these girls will enable us to assess the clinical consequences of transient thelarche.

The data that support the findings of this study are openly available in references 11 and 14.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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