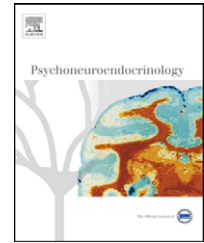




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# HPG-axis hormones during puberty: A study on the association with hypothalamic and pituitary volumes

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## KEYWORDS

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## Summary

**Objective:** During puberty, the hypothalamus–pituitary–gonadal (HPG) axis is activated, leading to increases in luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex steroids (testosterone and estradiol) levels. We aimed to study the association between hypothalamic and pituitary volumes and development of pubertal hormones in healthy pubertal children.

**Method:** Hormone levels of LH, FSH, estradiol (measured in urine) and testosterone (measured in saliva) were assessed in 85 healthy children (39 boys, 46 girls) between 10 and 15 years of age. Hypothalamic and pituitary gland volumes were segmented on high resolution structural MRI scans. Since sex hormone production is regulated in a sex-specific manner, associations between hormones, hypothalamus and pituitary were analyzed in boys and girls separately.

**Results:** LH, estradiol and testosterone levels all increased with age in both sexes, whereas FSH level did not. Pituitary volume also increased with age and explained 12%, 10% and 8% of the variance in female estradiol, testosterone and LH levels respectively. Corrected for age, pituitary volume explained 17% of FSH level in girls (not boys). Hypothalamic volume did not change with age and did not significantly explain variance in any hormonal level.

**Discussion:** Our study suggests that a larger pituitary volume is related to higher FSH production, but this association seems independent of pubertal development. The positive association between estradiol, LH and testosterone and pituitary volume is related to age-related pubertal development. With respect to the hypothalamus, we did not find convincing evidence for a larger structure to be involved in elevated hormonal production.

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## 1. Introduction

The hypothalamus and pituitary gland are key structures involved in mediating responses vital for maintaining homeostasis, including sleep, hunger, thirst, thermoregulation and sexual maturation (Tran et al., 2004). Sexual maturation is referred to as the period of puberty, which is characterized by (re)activation of the hypothalamus–pituitary–gonadal (HPG) axis. In brief, gonadotropin releasing hormone (GnRH) neurons in the hypothalamus induce the secretion of GnRH. GnRH and peripheral factors from the gonads regulate the production of gonadotropins LH and FSH from the pituitary gland (Marshall, 1995). The increase in LH and FSH during puberty induces the maturation of the gonads, leading to marked estradiol and testosterone secretion (Grumbach and Styne, 2003).

Recently, in children between 10 and 15 years of age, we demonstrated associations between age-related increases in female estradiol levels and cortical gray matter: increased gray matter density was observed in prefrontal and occipital areas, whereas decreased gray matter density was found in prefrontal, temporal and parietal areas (Peper et al., 2009a). Other HPG-axis hormones (testosterone, LH (Peper et al., 2009a) or FSH (unpublished data)) did not relate to cortical brain areas in this age group.

Animal studies found that increased levels of sex steroids (either exogenous or endogenous) and gonadotropins are associated with an increased degree of cell proliferation within the pituitary gland (Nolan and Levy, 2006a,b). Attempts have been made to indirectly relate human (sex) hormone production to morphology of the pituitary gland. For example, a more advanced phase of pregnancy (among others accompanied by increased estradiol activity) was associated with an enlarged pituitary (Gonzalez et al., 1988; Dinc et al., 1998). Furthermore, indirect evidence for an association between sex hormones and pituitary volumes comes from studies on sex differences (i.e. males and females display clear sex differences in hormonal profiles): during puberty, pituitary volume shows a growth spurt, which is more pronounced in girls than in boys (Takano et al., 1999). Also, a sex difference in pituitary height (Elster et al., 1990; Denk et al., 1999) and volume was found (MacMaster et al., 2007) which became apparent during adolescence (i.e. girls larger than boys) (Elster et al., 1990). But see (Fink et al., 2005) who did not report a sex difference in pituitary size or volume, although this was reported in pre-pubertal children.

Pituitary volume changes have also been related to increased activity of the hypothalamus–pituitary–adrenal (HPA) axis: for instance, psychosis has been associated with an enlarged pituitary volume (Pariante et al., 2004, 2005). It is suggested that the enlargement is due to an increase in the size and number of pituitary corticotrophin cells caused by the activation of the hormonal stress response (for review see Pariante, 2008).

In humans, the relation between HPG-axis hormones and hypothalamic volumes has been studied less well than pituitary volumes. Treatment with testosterone induced hypothalamic volume increases whereas androgen blocking medication decreased hypothalamic volumes (Hulshoff Pol et al., 2006). In healthy males (who evidently have higher testosterone levels than females), a larger hypothalamus

was found (Goldstein et al., 2001), providing indirect evidence for a positive association between testosterone and hypothalamic volume. Moreover, pathological conditions such as hypothalamic hamartoma's (i.e. benign tumors composed of neurons and astroglia located near or on the hypothalamus) have been associated with precocious puberty (Jung et al., 2005). Furthermore, increased hypothalamic volumes were observed in schizophrenia patients compared to healthy controls (Goldstein et al., 2007), a condition characterized by abnormal endocrinological profiling (Walker et al., 2008).

The aim of the current study was to explore the relation between levels of pubertal hormones (i.e. LH, FSH, testosterone and estradiol) and volume of the hypothalamus and pituitary gland. It was hypothesized that larger hypothalamic and pituitary gland volumes are both associated with higher hormonal production in both boys and girls.

## 2. Subjects and methods

### 2.1. Participants

The sample consisted of 85 healthy children between 10 and 15 years of age (mean 11.9 ( $\pm$ 1.1) years), including 39 boys and 46 girls recruited from the Netherlands Twin Register (Boomsma et al., 2006) (Table 1). These non-twin children and their younger twin-siblings took part in a larger study described elsewhere (Peper et al., 2008, 2009a,b; van Leeuwen et al., 2009). Exclusion criteria consisted of any major medical or psychiatric illness and participation in special education. Physical health and mental health were assessed with a medical history inventory. Parents and the participants themselves gave written informed consent to participate in the study. The study was approved by the Central Committee on Research involving Human Subjects (CCMO) of the Netherlands and was in agreement with the Declaration of Helsinki (Edinburgh amendments).

**Table 1** Characteristics of the sample.

	Boys		Girls	
	N	Mean (SD)	N	Mean (SD)
Age (y)	39	11.6 (1.0)	46	12.1 (1.2) <sup>*</sup>
Hypo (ml)	36	1.05 (.12)	40	1.01 (.09)
Pit (ml)	36	.53 (.13)	41	.58 (.14)
TB (ml)	39	1402.9 (92.7)	46	1304.4 (96.8) <sup>***</sup>
FSH (U/l)	37	.39 (.25)	40	.85 (.44) <sup>***</sup>
LH (U/l)	30	2.20 (2.19)	36	2.14 (2.30)
T (pmol/l)	29	70.8 (66.1)	38	58.7 (36.3)
E (pmol/l)	37	1027.0 (696.7)	35	2371.6 (1332.2) <sup>***</sup>
Tan A	38	1.63 (.75)	46	2.85 (.97) <sup>***</sup>
TanB	38	1.71 (.96)	46	2.83 (1.23) <sup>***</sup>
TanC	38	1.50 (.60)	—	—

Hypo = hypothalamus volume; Pit = pituitary gland volume; TB = total brain volume; T = testosterone; E/c = estradiol; Tan = Tanner stages.

<sup>\*</sup>  $p < .02$ .

<sup>\*\*\*</sup>  $p < .0001$  (sex differences, corrected for age).

## 2.2. MRI acquisition and processing

Structural magnetic resonance imaging (MRI) scans of the whole brain were made on a 1.5-T Achieva scanner (Philips, Best, the Netherlands).

A three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head ( $256 \times 256$  matrix, TE = 4.6 ms, TR = 30 ms, flip angle =  $30^\circ$ , 160–180 contiguous slices;  $1 \text{ mm} \times 1 \text{ mm} \times 1.2 \text{ mm}$  voxels, Field-of-View = 256 mm/70%) was acquired. The scans were coded to ensure blindness for subject identification. The T1-weighted images were automatically put into Talairach orientation (Talairach and Tournoux, 1988) without scaling, by registering them to a model brain in Talairach orientation. The translation and rotation parameters of this registration were then applied to the images (Maes et al., 1997). The T1-weighted images were corrected for field inhomogeneities using the N3 algorithm (Sled et al., 1998). Our automatic image processing pipeline was used for segmentation of total brain, gray and white matter of the cerebrum. The software included histogram analysis, mathematical morphology operations, and anatomical knowledge based rules to connect all voxels of interest, as was validated (Schnack et al., 2001) and described before (Peper et al., 2009b).

### 2.2.1. Hypothalamus and pituitary segmentation

Manual segmentations of the pituitary and hypothalamus were carried out by one single operator (JP) on the T1-weighted image in the coronal orientation. 10 MRIs were randomly chosen and cloned for intra-rater reliability as measured with the intraclass correlation coefficient (ICC) (Bartko and Carpenter, 1976). Intra-rater ICCs were .86 and .87 for hypothalamic and pituitary volumes respectively.

The hypothalamus consists of several different nuclei, which are difficult to distinguish on a 1.5 T MRI scan. Therefore the segment of the hypothalamus consisted of all separate nuclei (including the mammillary bodies), following the boundaries applied earlier (Hulshoff Pol et al., 2006; Koolschijn et al., 2008). In brief, the anterior border was defined as the first coronal slice where the anterior commissure was no longer continuous and the posterior border was identified as the slice where the mammillary bodies were completed. The lateral borders consisted of white matter. The segment was multiplied by the total brain segment to

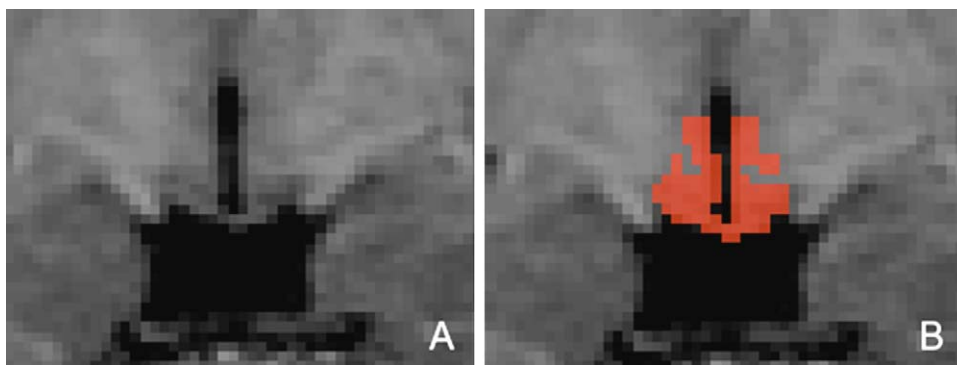
remove non-brain tissue (i.e. the 3rd ventricle) and by the inverse of the white matter segment to remove tissue belonging to the fornix. On average, the total hypothalamic segment consisted of ten 1.2-mm thick slices (Fig. 1). Separation of gray and white matter tissue was not possible in 7 subjects (2 boys); and two extreme outliers on hypothalamic volumes ( $>3 \text{ s.d.}$ ) were removed before analyses (1 boy), leading to a total number of 76 hypothalamic volumes. It must be noted that after including these extreme outliers of hypothalamic volumes in the analyses, the findings did not change. We preferred however to present the data without outliers.

The pituitary segment comprised both the anterior and posterior lobes. The sagittal view was used to locate the pituitary gland. The anterior border was the first (coronal) slice where the pituitary came into view. The posterior limit was formed by the dorsum sellae (within the sphenoid bone). Inferiorly, the sphenoid sinus limited the pituitary segment whereas cerebrospinal fluid and/or the infundibular stalk marked the borders superiorly. The surrounding blood vessels were used as lateral borders. This resulted in eight to ten 1.2-mm slices where the pituitary gland could be delineated (Fig. 2). Pituitary segmentation was not possible in 4 subjects (3 boys) due to motion artifacts, leading to a total number of pituitary segments in 81 subjects. Pituitary and hypothalamic volumes were normally distributed (Kolmogorov–Smirnov test:  $p = .98$  and  $p = .39$  respectively).

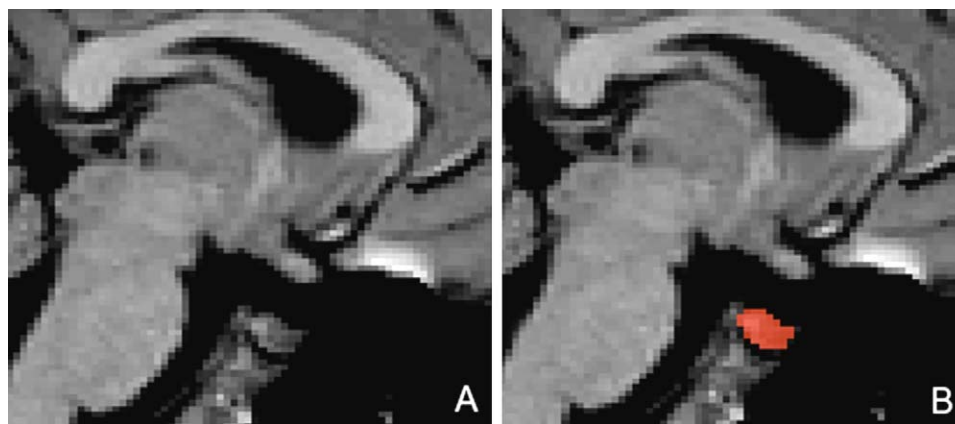
## 2.3. Hormonal measurements

LH, FSH and estradiol levels were determined in morning urine using highly sensitive immunometric assays (Luminiscence) (Architect, Abbott Laboratories, Abbott Park, IL, USA).

The detection limits of LH and FSH were .1 U/l and .11 U/l respectively with an intra-assay coefficient of variation (CV) of 3% and inter-assay CV of 6%. For estradiol, the intra-assay and inter-assay CVs were 5% and 10% respectively at levels  $>150 \text{ pmol/l}$  (lower) and  $<9000 \text{ pmol/l}$  (upper). Free testosterone levels were determined in first morning saliva (Competitive immunoassay (luminiscence), IBL Hamburg). The intra-assay and inter-assay CVs were below 12% at levels  $>11 \text{ pmol/l}$  (lower limit of detection). All hormonal data were collected on two consecutive days at consistent times directly after waking up, and the means of the two measurements



**Figure 1** Coronal view of the hypothalamus without (A) and with (B) segment. The hypothalamic segment consists on average of ten 1.2-mm slices. The segment is multiplied by the total brain segment to remove volume of 3rd ventricle and with the inverse of the white matter segment, to remove tissue of the Fornix.



**Figure 2** Sagittal view of the pituitary gland without (A) and with (B) segment. The pituitary segment consists on average of eight to ten 1.2-mm coronal slices and includes both the anterior and posterior parts.

were used in further analyses. Determination of hormone levels was carried out by the endocrinological laboratory of clinical chemistry of the VU Medical Center in Amsterdam, the Netherlands. FSH, LH and testosterone levels were not collected in 8 children (2 boys, 6 girls), and estradiol levels could not be determined in 3 girls. Furthermore, hormonal concentrations of a number of participants were below detection limits: 11 samples of LH (7 boys, 4 girls) and 10 samples of testosterone (8 boys, 2 girls). Thus, the remaining number of available hormone samples on which analyses were carried out consisted of 77 children with FSH (37 boys, 40 girls), 66 children with LH (30 boys, 36 girls), 67 children with testosterone (29 boys, 38 girls) and 72 children with estradiol samples (37 boys, 35 girls). A Kolmogorov–Smirnov test showed that hormonal levels were not normally distributed, therefore a log-transformation ( $\ln$ ) was applied, leading to a normal distribution of FSH ( $p = .43$ ), LH ( $p = .33$ ), testosterone ( $p < .35$ ) and estradiol ( $p = .74$ ).

Secondary sexual characteristics were additionally measured using a Tanner questionnaire (Marshall and Tanner, 1969, 1970), with 3 different scales: Tanner-A (stages 1–5) = penis development (boys), breast development (girls); Tanner-B (stages 1–6) = pubic hair development (both sexes); and Tanner-C (stages 1–4) = testicle development (boys). A regular menstrual cycle was present in 5 girls, but information on their menstrual phase during the time of MRI measurements was not available. None of the female participants used oral contraceptives.

#### 2.4. Statistical analysis

We first investigated whether HPG-axis hormones increased within the age-range of 10–15 years, using a linear regression analysis with age as a predictor variable. Second, the association between hypothalamic and pituitary volumes and hormonal levels was investigated using a regression analysis. The percentage of explained variance ( $R^2$ ) between hormonal and brain measures was also calculated within a regression analysis. Third, to consider whether hormone production independent of age, would be associated with pituitary and/or hypothalamic volumes, an additional regression analysis was carried out with age as a covariate (partial correlation). Finally, to investigate the effect of total brain volume

on possible associations between hormonal levels, pituitary or hypothalamic volumes, total brain volume was subsequently added as covariate.

Since hormone production is regulated in a sex-specific manner, all analyses were carried out in boys and girls separately. Results are reported statistically significant at a level of  $p < .05$ .

### 3. Results

On average, girls were older than boys ( $F_{(1,84)} = 5.79$ ,  $p = .02$ ), and when corrected for age, also in a more advanced stage of puberty (secondary sexual characteristics: Tanner-A:  $F_{(1,83)} = 37.67$ ,  $p < .00001$ ; Tanner-B:  $F_{(1,83)} = 14.63$ ,  $p = .0003$ ), as reported previously (Peper et al., 2009a). Pituitary and hypothalamic volumes were not significantly correlated in both sexes.

LH, estradiol (not in boys) and testosterone levels significantly increased with age, with correlation coefficients ranging between .41 and .60 (Table 2). In contrast, FSH levels in boys and girls did not increase with age. In close relation to this, LH, estradiol (not in boys) and testosterone levels were significantly related to development of secondary sexual characteristics (correlation coefficients ranging between .44 and .69), whereas FSH levels did not show an association with development of secondary sexual characteristics neither in girls nor in boys.

Pituitary volumes increased with age in girls ( $r = .45$ ;  $p < .002$ ) and boys ( $r = .35$ ;  $p < .04$ ), but hypothalamus volumes did not show an association with age.

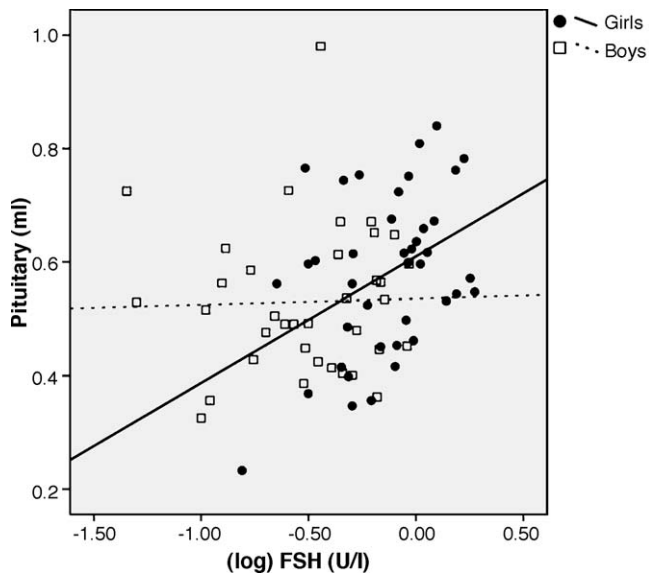
**Table 2** Correlation between HPG-axis hormones and age.

	Boys	Girls
FSH	.23	.07
LH	.51**	.60***
Testosterone	.59**	.41*
Estradiol	.24	.57**

\*  $p < .01$ .

\*\*  $p < .001$ .

\*\*\*  $p < .0001$ .



**Figure 3** Association between FSH level and pituitary volume in girls ( $N = 40$ ) and boys ( $N = 36$ ) between 10 and 15 years of age. Corresponding correlation coefficients obtained from a regression analysis are .40 ( $p < .01$ ) for girls and .03 (not significant) for boys. FSH levels are log-transformed (ln), with actual raw values ranging between .16–1.88 U/l (girls) and .04–.93 U/l (boys).

In girls, pituitary volume was significantly associated with FSH ( $r = .40$ ;  $R^2 = 16\%$ ) (Fig. 3) and with estradiol-levels ( $r = .40$ ;  $R^2 = 16\%$ ) (both  $p$ 's  $< .001$ ) (Table 3). The association between female pituitary volumes and testosterone or LH levels failed to reach statistical significance ( $p = .06$  and  $p = .08$  respectively). In boys, pituitary volume was not related to any hormone level. Male or female hypothalamic volumes did not show a significant relationship with any hormonal level (Table 3).

After correcting hormonal levels for age, only the association between pituitary volume and FSH level in girls remained significant ( $r = .41$ ;  $R^2 = 17\%$ ;  $p < .01$ ). In both sexes, pituitary volumes were not related to total brain volume, whereas in girls hypothalamic volume was positively correlated with total brain volume ( $r = .36$ ;  $p < .02$ ). Correcting the analyses for total brain volume did not affect the results in girls or boys.

**Table 3** Correlations ( $r$ ) and explained variance ( $R^2$ ) between brain volumes and HPG-axis hormones.

	Pituitary		Hypothalamus	
	Boys $r$ ( $R^2$ )	Girls $r$ ( $R^2$ )	Boys $r$ ( $R^2$ )	Girls $r$ ( $R^2$ )
FSH	.03	.40 (16%)**	.30 (9%) <sup>§</sup>	.06
LH	.24 (6%)	.28 (8%) <sup>‡</sup>	.07	-.03
T	.20 (4%)	.31 (10%) <sup>§</sup>	.33 (11%) <sup>‡</sup>	.07
E	.05	.40 (16%)*	-.16 (3%)	.02

$R^2$  is not shown when  $< 1\%$ . Correlations are uncorrected for age.

\*  $p < .05$ .

\*\*  $p < .01$ .

<sup>§</sup>  $p = .06$ .

<sup>‡</sup>  $p = .08$ .

To check for possible confounding effects of intra-individual variation of hormonal levels in the 5 (regularly) menstrual cycling girls, these subjects were removed from a subsequent analysis. Results did not show a substantial change of the data: a larger pituitary remained significantly related to higher levels of FSH and estradiol.

#### 4. Discussion

To our knowledge, this is the first study addressing the relation between HPG-axis hormone production during (early) puberty and hypothalamic and pituitary gland volumes. In healthy 10–15-year-old boys and girls, we found prominent age-related increases in levels of luteinizing hormone (LH), estradiol (not boys) and testosterone, but not in follicle stimulating hormone (FSH). In girls only, a larger pituitary volume was significantly related to increased production of FSH and estradiol, explaining 16% and 12% of their variance respectively. FSH levels did not increase in this age range. Thus, after correcting for age effects, only the association between FSH levels and pituitary volumes remained significant. Indeed, other studies reported steep increases of LH, testosterone and estradiol with advanced puberty, but not of FSH, which was found to increase only moderately during the course of puberty (Maruyama et al., 1987; Dunkel et al., 1992; Demir et al., 1995, 1996; Albertsson-Wikland et al., 1997). On top of that, gradual FSH increases are already ongoing several years before the onset of puberty in both sexes (Raivio and Dunkel, 2002). Therefore, our observations suggest that pubertal increases of LH, estradiol and testosterone levels are associated with a larger pituitary gland, whereas the association between FSH and pituitary volume is relatively independent of pubertal development.

We found that in girls, pituitary volume explained twice as much variance in FSH levels than in LH levels (16% versus 8%). An explanation for this difference might be that LH and FSH are differentially influenced by other regulatory factors. Besides GnRH release from the hypothalamus, the regulatory peptides Inhibin-A (in girls) and Inhibin-B (in both boys and girls) are involved in regulating LH and FSH release (Bergada et al., 2001; Foster et al., 2004). Inhibins are produced from Sertoli cells from the testes and from granulosa cells of ovarian follicles. During puberty, Inhibin-B is more strongly related to FSH than to LH release (or testosterone and estradiol) (Sehested et al., 2000; Chada et al., 2003a,b). Moreover, it has been reported that FSH is for a larger part independent of GnRH-release from the hypothalamus than LH (Apter et al., 1993). Thus, LH and FSH levels are differentially regulated by distinct peptides.

Although LH and FSH are secreted directly from the pituitary gland, its volume could only explain a moderate amount of variance in FSH and LH levels (16% and 8% in girls and 0% and 6% in boys respectively). In our study, the pituitary segment contained both the anterior and posterior lobe, due to inconsistent and difficult visualization of the two distinct lobes. Besides LH and FSH secretion, the anterior lobe (or adenohypophysis) is involved in the regulation of corticotropin (ACTH), thyroid stimulating hormone (TSH), prolactin, growth hormone (GH) and endorphins, whereas the posterior lobe (or neurohypophysis) is involved in secretion of oxytocin and vasopressin (Tran et al., 2004). Thus, other (endocrine)

factors than gonadotropins might be involved in determining/influencing the volume of the pituitary. For example, a larger pituitary predicted increased production of GH (Nagel et al., 1997; Tillmann et al., 2000). Moreover, increased pituitary volumes were found to be related to first-episode psychosis (Pariante et al., 2004; Mondelli et al., 2008; Takahashi et al., 2009), depression (MacMaster et al., 2008) and post-traumatic stress disorder (Thomas and De Bellis, 2004): conditions characterized by elevated levels of ACTH and cortisol through HPA-axis hyperactivity (Yehuda et al., 1991; Yehuda, 2001; Pariante and Lightman, 2008; Walker et al., 2008).

We could only demonstrate a significant association between pituitary volume and FSH levels in girls. An explanation for this sex difference could be that in boys, inhibin-B levels are increasing earlier and faster than in girls (Bergada et al., 2001; Raivio and Dunkel, 2002), thus FSH levels in boys are inhibited more pronounced, possibly by other (non-pituitary) related factors, such as inhibins. Indeed, in our sample boys showed significantly lower levels of FSH than girls (corrected for the age-difference between the sexes) (see Table 1). Another more general explanation for not being able to demonstrate a (significant) association between HPG-axis hormones and pituitary and/or hypothalamus volumes in boys, might be their less advanced pubertal stage within the age-range studied here. That is, if our observations in girls (age-related increase in hormonal levels is related to enlarged pituitary volumes) imply that the pituitary needs to have a certain volume to produce a certain level of hormones, than it can be expected that this association in boys becomes apparent at a somewhat later pubertal stage. Indeed, peak levels of testosterone production in boys are found at (genital) Tanner stage 4 (Butler et al., 1989) which is on average reached at 13 years of age (Marshall and Tanner, 1970) (note that mean age of the male sample was 11.6 years). Although a substantial part of the female sample had already reached mid-puberty (mean Tanner stage 2.8 out of 5 and 6 scales), maximal sex hormone production will be reached after a few more years. The necessity of an older sample to interpret effects of sex hormone production is also underscored by (MacMaster et al., 2007), who demonstrated sex differences in pituitary volumes within 14–17-year-old subjects, whereas in younger participants this sexual dimorphism could not be found.

There are some other limitations in our study that need to be addressed. Next to being relatively young with respect to pubertal development, the group of boys consisted of 29 boys in which reliable testosterone measurements could be carried out. Thus, a lack of power might have caused the association between testosterone level and hypothalamus volume failing to reach statistical significance. Animal data indeed suggest that circulating androgens are able to enlarge soma size of neurons in the sexual dimorphic nucleus of the pre-optic area (SDN-POA) (Dugger et al., 2008). Moreover, post mortem studies in humans have reported clear structural sex differences in distinct nuclei of the hypothalamus, including the SDN-POA (Swaab and Fliers, 1985) and the suprachiasmatic nucleus (SCN) (Fernandez-Guasti et al., 2000) (larger in males), possibly pointing at an increasing effect of testosterone on these hypothalamic nuclei. Another limitation concerns segmenting of the hypothalamus. Although intra-rater reliability of hypothalamic segments

was very high (ICC = .86) and scans of bad quality were removed from the analysis, when using volumetric MRI the exact delineation of the hypothalamic region borders is sometimes difficult. The hypothalamus is surrounded by tissues consisting of a mixture of gray- and white-matter, which at times are not easily discernable from the actual hypothalamus. Thus, a detailed atlas of basal forebrain and diencephalic topography was used for an accurate segmentation of the hypothalamus using MRI (Niewenhuys et al., 1988). Our MRI-based hypothalamic segments consisted of all nuclei together, as 1.5 T MRI scans do not allow for (reliable) segmentation of these separate hypothalamic nuclei. Not being able to detect a significant relation between hypothalamic volumes and hormonal levels might possibly be explained by segmentation-related issues described above. For example, we might have overlooked subtle changes in separate hypothalamic nuclei.

The secretion of HPG-axis related hormones is the result of a complex interplay between different factors. It is under the control of negative steroid hormone feedback from the gonads (Roseweir and Millar, 2009): estrogen, when reaching a certain threshold, at the level of the hypothalamus inhibits the GnRH-pulse frequency and at the level of the pituitary decreases responsiveness to GnRH production (Hayes et al., 2000). In our sample we observed a positive association between pituitary volume and all hormonal levels (Table 3). Considering the negative feedback system of the HPG-axis, this observation seems counterintuitive. However, it has been put forward that the negative feedback loop is not closed until mid-to-late puberty (Andersson et al., 1997). Thus, in our relatively early pubertal sample (especially in boys), higher sex steroid production probably still does not decrease hypothalamic GnRH secretion and/or pituitary LH and FSH secretion.

Evidently, using our current correlational design, it is not possible to draw any conclusions on the direction of the observed association between HPG-axis hormones and brain structures. Since LH and FSH are directly produced from the pituitary gland, it seems reasonable to suggest that a larger gland leads to increased LH/FSH production. However, as mentioned earlier, gonadotropins stimulate the gonadal organs through a feedback mechanism, that in turn act on the hypothalamus and pituitary (Roseweir and Millar, 2009). For example, it has been found that glial cells in the arcuate nucleus of the hypothalamus regulate GnRH activity (Garcia-Segura et al., 2008). On the other hand, gonadal hormones at puberty can in turn activate reorganization of GnRH neurons within the hypothalamus and change pituitary and gonadal activity (Garcia-Segura et al., 2008). Based on these reciprocal functions, it is therefore difficult to indicate the source of our reported associations between the pituitary gland and hormonal levels.

In conclusion, our study suggests that at least in girls, a larger pituitary volume is related to increased FSH production, but this association seems independent of pubertal development. On the other hand, the positive association between estradiol, LH and testosterone and pituitary volume is related to age-related pubertal development. With respect to the hypothalamus, we did not find compelling evidence for a larger structure to be involved in elevated hormonal production. To be able to investigate maximal influences of sex steroid levels on the developing brain, future studies should include more advanced pubertal children.

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## Conflict of interest

The authors declare no competing financial interests.

## References

- Albertsson-Wikland, K., Rosberg, S., Lannering, B., Dunkel, L., Selstam, G., Norjavaara, E., 1997. Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. *J. Clin. Endocrinol. Metab.* 82, 541–549.
- Andersson, A.M., Juul, A., Petersen, J.H., Muller, J., Groome, N.P., Skakkebaek, N.E., 1997. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J. Clin. Endocrinol. Metab.* 82, 3976–3981.
- Apter, D., Butzow, T.L., Laughlin, G.A., Yen, S.S., 1993. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J. Clin. Endocrinol. Metab.* 76, 940–949.
- Bartko, J.J., Carpenter Jr., W.T., 1976. On the methods and theory of reliability. *J. Nerv. Ment. Dis.* 163, 307–317.
- Bergada, I., Bergada, C., Campo, S., 2001. Role of inhibins in childhood and puberty. *J. Pediatr. Endocrinol. Metab.* 14, 343–353.
- Boomsma, D.I., de Geus, E.J., Vink, J.M., Stubbe, J.H., Distel, M.A., Hottenga, J.J., Posthuma, D., van Beijsterveldt, T.C., Hudziak, J.J., Bartels, M., Willemsen, G., 2006. Netherlands Twin Register: from twins to twin families. *Twin Res. Hum. Genet.* 9, 849–857.
- Butler, G.E., Walker, R.F., Walker, R.V., Teague, P., Riad-Fahmy, D., Ratcliffe, S.G., 1989. Salivary testosterone levels and the progress of puberty in the normal boy. *Clin. Endocrinol. (Oxf.)* 30, 587–596.
- Chada, M., Prusa, R., Bronsky, J., Kotaska, K., Sidlova, K., Pechova, M., Lisa, L., 2003a. Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol. Res.* 52, 45–51.
- Chada, M., Prusa, R., Bronsky, J., Pechova, M., Kotaska, K., Lisa, L., 2003b. Inhibin B, follicle stimulating hormone, luteinizing hormone, and estradiol and their relationship to the regulation of follicle development in girls during childhood and puberty. *Physiol. Res.* 52, 341–346.
- Demir, A., Dunkel, L., Stenman, U.H., Voutilainen, R., 1995. Age-related course of urinary gonadotropins in children. *J. Clin. Endocrinol. Metab.* 80, 1457–1460.
- Demir, A., Voutilainen, R., Juul, A., Dunkel, L., Alfthan, H., Skakkebaek, N.E., Stenman, U.H., 1996. Increase in first morning voided urinary luteinizing hormone levels precedes the physical onset of puberty. *J. Clin. Endocrinol. Metab.* 81, 2963–2967.
- Denk, C.C., Onderoglu, S., Ilgi, S., Gurcan, F., 1999. Height of normal pituitary gland on MRI: differences between age groups and sexes. *Okajimas Folia Anat. Jpn.* 76, 81–87.
- Dinc, H., Esen, F., Demirci, A., Sari, A., Resit Gumele, H., 1998. Pituitary dimensions and volume measurements in pregnancy and post partum. *MR assessment. Acta Radiol.* 39, 64–69.
- Dugger, B.N., Morris, J.A., Jordan, C.L., Breedlove, S.M., 2008. Gonadal steroids regulate neural plasticity in the sexually dimorphic nucleus of the preoptic area of adult male and female rats. *Neuroendocrinology* 88, 17–24.
- Dunkel, L., Alfthan, H., Stenman, U.H., Selstam, G., Rosberg, S., Albertsson-Wikland, K., 1992. Developmental changes in 24-hour profiles of luteinizing hormone and follicle-stimulating hormone from prepuberty to midstages of puberty in boys. *J. Clin. Endocrinol. Metab.* 74, 890–897.
- Elster, A.D., Chen, M.Y., Williams 3rd, D.W., Key, L.L., 1990. Pituitary gland: MR imaging of physiologic hypertrophy in adolescence. *Radiology* 174, 681–685.
- Fernandez-Guasti, A., Kruijver, F.P., Fodor, M., Swaab, D.F., 2000. Sex differences in the distribution of androgen receptors in the human hypothalamus. *J. Comp. Neurol.* 425, 422–435.
- Fink, A.M., Vidmar, S., Kumbala, S., Pedreira, C.C., Kanumakala, S., Williams, C., Carlin, J.B., Cameron, F.J., 2005. Age-related pituitary volumes in prepubertal children with normal endocrine function: volumetric magnetic resonance data. *J. Clin. Endocrinol. Metab.* 90, 3274–3278.
- Foster, C.M., Olton, P.R., Racine, M.S., Phillips, D.J., Padmanabhan, V., 2004. Sex differences in FSH-regulatory peptides in pubertal age boys and girls and effects of sex steroid treatment. *Hum. Reprod.* 19, 1668–1676.
- Garcia-Segura, L.M., Lorenz, B., DonCarlos, L.L., 2008. The role of glia in the hypothalamus: implications for gonadal steroid feedback and reproductive neuroendocrine output. *Reproduction* 135, 419–429.
- Goldstein, J.M., Seidman, L.J., Horton, N.J., Makris, N., Kennedy, D.N., Caviness Jr., V.S., Faraone, S.V., Tsuang, M.T., 2001. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cereb. Cortex* 11, 490–497.
- Goldstein, J.M., Seidman, L.J., Makris, N., Ahern, T., O'Brien, L.M., Caviness Jr., V.S., Kennedy, D.N., Faraone, S.V., Tsuang, M.T., 2007. Hypothalamic abnormalities in schizophrenia: sex effects and genetic vulnerability. *Biol. Psychiatry* 61, 935–945.
- Gonzalez, J.G., Elizondo, G., Saldivar, D., Nanez, H., Todd, L.E., Villarreal, J.Z., 1988. Pituitary gland growth during normal pregnancy: an in vivo study using magnetic resonance imaging. *Am. J. Med.* 85, 217–220.
- Grumbach, M.M., Styne, D.M., 2003. Puberty ontogeny, neuroendocrinology, physiology and disorders. In: Larsen, P.R., Kronenberg, H.M., Melmed, S., Polonsky, K.S. (Eds.), *Williams Textbook of Endocrinology*. Elsevier, New York, pp. 1115–1286.
- Hayes, F.J., Seminara, S.B., Decruz, S., Boepple, P.A., Crowley Jr., W.F., 2000. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. *J. Clin. Endocrinol. Metab.* 85, 3027–3035.
- Hulshoff Pol, H.E., Cohen-Kettenis, P.T., van Haren, N.E., Peper, J.S., Brans, R.G., Cahn, W., Schnack, H.G., Gooren, L.J.G., Kahn, R.S., 2006. Changing your sex changes your brain: influences of testosterone and estrogen on adult human brain structure. *Eur. J. Endocrinol.* 155, s107–s114.
- Jung, H., Parent, A.S., Ojeda, S.R., 2005. Hypothalamic hamartoma: a paradigm/model for studying the onset of puberty. *Endocr. Dev.* 8, 81–93.
- Koolschijn, P.C., van Haren, N.E., Hulshoff Pol, H.E., Kahn, R.S., 2008. Hypothalamus volume in twin pairs discordant for schizophrenia. *Eur. Neuropsychopharmacol.* 18, 312–315.
- MacMaster, F.P., Keshavan, M., Mirza, Y., Carrey, N., Upadhyaya, A.R., El-Sheikh, R., Buhagiar, C.J., Taormina, S.P., Boyd, C., Lynch, M., Rose, M., Ivey, J., Moore, G.J., Rosenberg, D.R., 2007. Development and sexual dimorphism of the pituitary gland. *Life Sci.* 80, 940–944.
- MacMaster, F.P., Leslie, R., Rosenberg, D.R., Kusumakar, V., 2008. Pituitary gland volume in adolescent and young adult bipolar and unipolar depression. *Bipolar Disord.* 10, 101–104.

- Maes, F., Collignon, A., Vandermeulen, D., Marchal, G., Suetens, P., 1997. Multimodality image registration by maximization of mutual information. *IEEE Trans. Med. Imaging* 16, 187–198.
- Marshall, J.C., 1995. Regulation of gonadotropin secretion. In: DeGroot, L.J. (Ed.), *Endocrinology*. Saunders, Philadelphia.
- Marshall, W.A., Tanner, J.M., 1969. Variations in pattern of pubertal changes in girls. *Arch. Dis. Child* 44, 291–303.
- Marshall, W.A., Tanner, J.M., 1970. Variations in the pattern of pubertal changes in boys. *Arch. Dis. Child* 45, 13–23.
- Maruyama, Y., Aoki, N., Suzuki, Y., Ohno, Y., Imamura, M., Saika, T., Sinohara, H., Yamamoto, T., 1987. Sex-steroid-binding plasma protein (SBP), testosterone, oestradiol and dehydroepiandrosterone (DHEA) in prepuberty and puberty. *Acta Endocrinol. (Copenh.)* 114, 60–67.
- Mondelli, V., Dazzan, P., Gabilondo, A., Tournikioti, K., Walshe, M., Marshall, N., Schulze, K.K., Murray, R.M., McDonald, C., Pariante, C.M., 2008. Pituitary volume in unaffected relatives of patients with schizophrenia and bipolar disorder. *Psychoneuroendocrinology* 33, 1004–1012.
- Nagel, B.H., Palmbach, M., Petersen, D., Ranke, M.B., 1997. Magnetic resonance images of 91 children with different causes of short stature: pituitary size reflects growth hormone secretion. *Eur. J. Pediatr.* 156, 758–763.
- Niewenhuis, R., Voogd, J., Van Huijzen, C., 1988. *The Human Central Nervous System: A Synopsis and Atlas*, 3rd ed. Springer-Verlag, Berlin.
- Nolan, L.A., Levy, A., 2006a. A population of non-luteinising hormone/non-adrenocorticotrophic hormone-positive cells in the male rat anterior pituitary responds mitotically to both gonadectomy and adrenalectomy. *J. Neuroendocrinol.* 18, 655–661.
- Nolan, L.A., Levy, A., 2006b. The effects of testosterone and oestrogen on gonadectomised and intact male rat anterior pituitary mitotic and apoptotic activity. *J. Endocrinol.* 188, 387–396.
- Pariante, C.M., 2008. Pituitary volume in psychosis: the first review of the evidence. *J. Psychopharmacol.* 22, 76–81.
- Pariante, C.M., Dazzan, P., Danese, A., Morgan, K.D., Brudaglio, F., Morgan, C., Fearon, P., Orr, K., Hutchinson, G., Pantelis, C., Velakoulis, D., Jones, P.B., Leff, J., Murray, R.M., 2005. Increased pituitary volume in antipsychotic-free and antipsychotic-treated patients of the AESop first-onset psychosis study. *Neuropsychopharmacology* 30, 1923–1931.
- Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* 31, 464–468.
- Pariante, C.M., Vassilopoulou, K., Velakoulis, D., Phillips, L., Soulsby, B., Wood, S.J., Brewer, W., Smith, D.J., Dazzan, P., Yung, A.R., Zervas, I.M., Christodoulou, G.N., Murray, R., McGorry, P.D., Pantelis, C., 2004. Pituitary volume in psychosis. *Br. J. Psychiatry* 185, 5–10.
- Peper, J.S., Brouwer, R.M., Schnack, H.G., van Baal, G.C., van Leeuwen, M., van den Berg, S.M., Delemarre-Van de Waal, H.A., Boomsma, D.I., Kahn, R.S., Hulshoff Pol, H.E., 2008. Cerebral white matter in early puberty is associated with luteinizing hormone concentrations. *Psychoneuroendocrinology* 33, 909–915.
- Peper, J.S., Brouwer, R.M., Schnack, H.G., van Baal, G.C., van Leeuwen, M., van den Berg, S.M., Delemarre-Van de Waal, H.A., Boomsma, D.I., Kahn, R.S., Hulshoff Pol, H.E., 2009a. Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology* 34, 332–342.
- Peper, J.S., Schnack, H.G., Brouwer, R.M., vanBaal, G.C.M., Pjetri, E., Székely, E., Van Leeuwen, M., van den Berg, S.M., Collins, D.L., Evans, A.C., Boomsma, D.I., Kahn, R.S., HulshoffPol, H.E., 2009b. Heritability of global and regional brain structure at the onset of puberty: a magnetic resonance imaging study in 9-year old twin-pairs. *Hum. Brain Mapp.* 30, 2184–2196.
- Raivio, T., Dunkel, L., 2002. Inhibins in childhood and puberty. *Best Pract. Res. Clin. Endocrinol. Metab.* 16, 43–52.
- Roseweir, A.K., Millar, R.P., 2009. The role of kisspeptin in the control of gonadotrophin secretion. *Hum. Reprod. Update* 15, 203–212.
- Schnack, H.G., Hulshoff Pol, H.E., Baare, W.F., Staal, W.G., Vieregger, M.A., Kahn, R.S., 2001. Automated separation of gray and white matter from MR images of the human brain. *Neuroimage* 13, 230–237.
- Sehested, A., Juul, A.A., Andersson, A.M., Petersen, J.H., Jensen, T.K., Muller, J., Skakkebaek, N.E., 2000. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *J. Clin. Endocrinol. Metab.* 85, 1634–1640.
- Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans. Med. Imaging* 17, 87–97.
- Swaab, D.F., Fliers, E., 1985. A sexually dimorphic nucleus in the human brain. *Science* 228, 1112–1115.
- Takahashi, T., Suzuki, M., Velakoulis, D., Lorenzetti, V., Soulsby, B., Zhou, S.Y., Nakamura, K., Seto, H., Kurachi, M., Pantelis, C., 2009. Increased pituitary volume in schizophrenia spectrum disorders. *Schizophr. Res.* 108, 114–121.
- Takano, K., Utsunomiya, H., Ono, H., Ohfu, M., Okazaki, M., 1999. Normal development of the pituitary gland: assessment with three-dimensional MR volumetry. *AJNR Am. J. Neuroradiol.* 20, 312–315.
- Talairach, J., Tournoux, P., 1988. *Co-planar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. Thieme Medical Publishers, New York.
- Thomas, L.A., De Bellis, M.D., 2004. Pituitary volumes in pediatric maltreatment-related posttraumatic stress disorder. *Biol. Psychiatry* 55, 752–758.
- Tillmann, V., Tang, V.W., Price, D.A., Hughes, D.G., Wright, N.B., Clayton, P.E., 2000. Magnetic resonance imaging of the hypothalamic–pituitary axis in the diagnosis of growth hormone deficiency. *J. Pediatr. Endocrinol. Metab.* 13, 1577–1583.
- Tran, P.V., Savage, J.J., Ingraham, H.A., Rhodes, S.J., 2004. Molecular genetics of hypothalamic–pituitary axis development. In: Pescovitz, O.H., Eugster, E.A. (Eds.), *Pediatric Endocrinology, Mechanisms, Manifestations and Management*. Lippincott Williams & Wilkins, Philadelphia, pp. 63–79.
- van Leeuwen, M., Peper, J.S., van den Berg, S.M., Brouwer, R.M., Hulshoff Pol, H.E., Kahn, R.S., Boomsma, D.I., 2009. A genetic analysis of brain volumes and IQ in children. *Intelligence* 37, 181–191.
- Walker, E., Mittal, V., Tessner, K., 2008. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu. Rev. Clin. Psychol.* 4, 189–216.
- Yehuda, R., 2001. Biology of posttraumatic stress disorder. *J. Clin. Psychiatry* 62 (S17), 41–46.
- Yehuda, R., Giller, E.L., Southwick, S.M., Lowy, M.T., Mason, J.W., 1991. Hypothalamic–pituitary–adrenal dysfunction in posttraumatic stress disorder. *Biol. Psychiatry* 30, 1031–1048.