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Sex Differences in the Effect of Puberty on Hippocampal Morphology

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Abstract

Objective—Puberty is the defining process of adolescence, and is accompanied by divergent trajectories of behavior and cognition for males and females. Here we examine whether sex differences exist in the effect of puberty on the morphology of the hippocampus and amygdala.

Method—T1-weighted structural neuroimaging was performed in a sample of 524 pre- or postpubertal adolescents ages 10–22. Hippocampal and amygdala volume and shape were quantified using FSL's FIRST procedure and scaled by intracranial volume. The effects on regional volume of age, sex, puberty, and their interactions were examined using linear regression. Postpubertal sex differences were examined using a vertex analysis.

Results—Prepubertal males and females had similar hippocampal volumes, whereas postpubertal females had significantly larger bilateral hippocampi, resulting in a significant puberty-by-sex interaction even when controlling for age and age-by-sex. This effect was regionally specific and was not apparent in the amygdala. Vertex analysis revealed that postpubertal differences were most prominent in the lateral aspect of the hippocampus bilaterally, corresponding to the CA1 subfield.

Conclusions—These results establish that there are regionally specific sex differences in the effect of puberty on the hippocampus. These findings are relevant for the understanding of psychiatric disorders that have both hippocampal dysfunction and prominent gender disparities during adolescence.

Keywords

adolescence; development; hippocampus; magnetic resonance imaging (MRI); puberty

INTRODUCTION

Adolescence is a period of marked changes in behavior, emotion, and cognition.^{1–3} The central biological process of the adolescent epoch is puberty, which is associated with evolving physical and psychological differences between males and females.⁴ Well-documented sex differences in brain structure also emerge during adolescence.^{5–9} The medial temporal lobe (MTL) structures of the hippocampus and amygdala are two regions where sex differences are most frequently reported.^{10,11} For example, Giedd *et al.* previously reported an increase in hippocampal volume in adolescent females but not males.¹⁰ Such effects are particularly notable given that in general gray matter volume declines substantially during adolescence,¹² and raises the possibility that specific patterns may be due to regionally specific actions of sex steroids. However, at present it is unknown whether sex differences in hippocampus and amygdala volume are specifically due to the impact of puberty. Adolescence does not equate to puberty,⁴ and other factors such as in-utero exposure to gonadal hormones and later environmental influences might result in the appearance of nonpubertal sex differences during adolescence.^{13–15}

Data from animal models suggests that sex differences in the impact of puberty on medial temporal lobe structures may exist. During puberty, circulating levels of gonadal hormones rise, with circulating estrogen reaching levels 2–4× higher in females than males (depending on the phase of the menstrual cycle).¹⁶ Importantly, both the hippocampus and amygdala have a high density of receptors for gonadal hormones.¹⁷ Prior research suggests that estrogen increases hippocampal dendritic spine density, synapse formation, axonal sprouting, and enhances long-term potentiation in the hippocampus.^{17–20} Estrogens have

also been linked to neurogenesis in the medial temporal lobe.^{21,22} It should be noted, however, that aromatase converts androgens into estrogens in males; aromatase activity is induced by testosterone and in animal models aromatase-derived estrogens influence the development of normal male social behaviors.^{23–25} Recent data suggests further complexity: androgen metabolites can have potent action at the estrogen receptor,²⁶ and intracellular binding sites for both androgens and estrogens have been linked to the complex regulation of the apoptotic cascade.²⁷ However, on the balance the above evidence suggests that estrogens may promote neurogenesis and synapse formation, and gives rise to the possibility that estrogens may mitigate the process of gray matter loss in specific tissues such as the hippocampus.

As noted above, studies in adolescent humans have frequently reported sex differences in the hippocampus and amygdala.^{10,11} However, subsequent efforts to attribute MTL differences to the effects of puberty have produced heterogeneous results. Blanton *et al.* (2012) recently examined the effect of puberty on amygdala and hippocampus volumes in a sample of 54 girls and found that puberty was related to diminished volume of both the hippocampus and amygdala. Bramen *et al.* (2011) reported similar results in females (n=48), but additionally found an opposite effect in males (n=32), where the transition to puberty was associated with larger amygdala and hippocampus volumes. In contrast, Neufang *et al.* (2009) found that puberty was associated with a main effect of both a smaller bilateral hippocampus and also a larger left amygdala in both males and females (n=23 each).

However, it should be noted that because age and puberty are highly correlated, small studies have not been sufficiently powered to disentangle their collinear effects and often have not modeled age and puberty together (the study by Bramen *et al.*, 2011, is one exception). Understanding the specific impact of puberty on the MTL development is important as abnormalities of hippocampus and amygdala structure and function have been linked to a wide range of psychiatric disorders that often begin following puberty and have strong gender disparities, including depression, anxiety disorders, and schizophrenia.^{28–31}

Utilizing a large sample of pre- and postpubertal adolescents studied as part of the Philadelphia Neurodevelopmental Cohort (n=524),³² we investigated sex differences in the effects of puberty on the MTL. We hypothesized that MTL volumes would be similar in prepubertal males and females, and diverge in the postpubertal period. Critically, by virtue of the large sample size available, we were able to dissociate sex differences in the effects of puberty from general effects of age, sex, and their interaction by modeling all variables jointly in the same model. Additionally, a vertex-based analysis was used to provide further delineation of local patterns of sex-specific effects of puberty.

METHOD

Participants

The present report concerns the Philadelphia Neurodevelopmental Cohort, which is an ongoing collaboration between the Brain Behavior Laboratory at the University of Pennsylvania (Penn) and the Center for Applied Genomics at Children's Hospital of Philadelphia (CHOP). Study procedures were reviewed and approved by the Institutional Review Boards of both Penn and CHOP. The target population-based sample is of 10,000 youths who presented to the CHOP network for a pediatric visit and volunteered to participate in genomic studies of complex pediatric disorders. All subjects or their parent or guardian provided informed consent and minors provided assent. From this pool of subjects, 1,445 were randomly selected for neuroimaging.³² The present analysis focuses on the 733 subjects of this cohort that were rated as either pre- or postpubertal according to a self-assessment (see below). Of these 733 subjects who were eligible for inclusion in the present

analysis, 135 subjects were excluded due to a history that suggested a potential abnormality of brain development. As in prior analyses of normal brain development in this sample,³³ subjects were excluded from this analysis if they had a history of inpatient psychiatric hospitalization (n=28), a history of a medical disorder that could impact brain function (n=35; e.g., sickle cell, HIV, past or current malignancy, epilepsy), were taking medications with potentially psychotropic effects (n=85), or had an incidentally-encountered abnormality of brain structure (n=9); subjects frequently met more than one exclusion criteria. Of the remaining 598 subjects, 74 subjects failed imaging QA procedures (detailed below), resulting in a final sample of 524 subjects (Table 1).

Assessment of Secondary Sex Characteristics and Selection of the of Pre-/Postpubertal Subsample

For the present large-scale community-based study, we used an abbreviated version of a self-report measure of pubertal status,³⁴ which was computerized and self-administered. Following general instructions, each participant age 10 and older privately viewed pictorial schematic representations, accompanied by text descriptions, of the five Tanner stages of pubic hair growth appropriate for his/her sex, and rated his/her own development on the scale from 1–5.³⁵ Prior validation studies have compared such self-report with Tanner staging conducted by a physician, and found that correlations between physician ratings and self-reported pubic hair ratings were .81 for girls and .63 for boys.³⁴ Due to such known limitations of Tanner stage self-report measures, in the present analysis we focused on subjects who were clearly identified as pre- or postpubertal. As in prior studies, ratings of 1 or 2 were considered prepubertal;^{36,37} a rating of 5 was considered postpubertal. Subjects with a mid-pubertal rating of 3 or 4 were not included in the present analysis.

Image Acquisition

Structural brain imaging was performed on all subjects using the same imaging sequence (magnetization-prepared, rapid acquisition gradient-echo T1-weighted structural image) on the same scanner (Siemens Tim Trio 3 Tesla, Erlangen, Germany; 32 channel head coil). Image acquisition parameters were: repetition time (TR) 1810 ms, time to echo (TE) 3.51 ms, field of view (FOV) 180 × 240 mm, matrix 256 × 192, 160 slices, TI 1100 ms, flip angle 9 degrees, effective voxel resolution of 0.9 × 0.9 × 1 mm. Prior to scanning, in order to acclimate subjects to the MRI environment, a mock scanning session was conducted using a decommissioned MRI scanner and head coil. A trained technician performed scanner QA regularly in order to monitor scanner stability.

Image Processing and Quality Assurance

All image processing was conducted using tools that are part of FSL.³⁸ Hippocampal volumes were estimated using the FIRST procedure (Figure 1).³⁹ Following registration to a template using settings optimized for subcortical structures, FIRST fits a deformable mesh of the hippocampus to each subject's T1 image within a Bayesian framework. As males are known to have larger head sizes than females,⁸ all hippocampal volumes were scaled by intracranial volume as estimated with FSL's SIENAX procedure. Subject level processing was implemented as part of an integrated processing and data management pipeline using NiPype⁴⁰ and PyXNAT⁴¹ within a custom XNAT database.⁴²

All hippocampal volumes underwent a rigorous quality assurance procedure, based in part on the standards outlined by the ENIGMA consortium.⁴³ Specifically, all images were visually inspected at three stages: the input image, following optimized subcortical registration, and following subcortical segmentation. Images with incomplete acquisition or degraded quality (due to motion or scanner artifact) were excluded from analysis. Additionally, hippocampal and amygdalar segmentations were evaluated individually as part

of the general QA workflow described above and also during a final step where outliers were re-examined. In this process, separate distributions for all volumes considered (both right and left hippocampus and amygdala) were constructed for using both ICV-corrected and raw volumes; outliers >2.5 S.D. from the mean were flagged and re-inspected for segmentation errors. Furthermore, laterality distributions were constructed separately for the hippocampus and amygdala (left volume – right volume / ([left + right volume] * 0.5)), and outliers were similarly flagged for re-inspection. This comprehensive QA procedure led to consistently high data quality despite the large size of the dataset. Subjects were required to have passed QA for all 4 regions (e.g., both right and left amygdala and hippocampus) to be included in the present analysis; as noted above, 74 subjects failed QA and were excluded from all analyses.

Statistical Analyses

We examined the effects of puberty while controlling for the effects of sex, age, and their interaction. Accordingly, age, sex, and an age by sex interaction term were included in a linear model in order to isolate specific effects of puberty above and beyond effects of age and sex. However, it should be noted that the age by sex and puberty by sex interaction terms were not significant in either the left or right amygdala and were therefore removed from the final model. Thus, the final models used were:

$$\begin{aligned}\text{Hippocampus volume} &= \text{intercept} + \text{age} + \text{sex} + \text{age} * \text{sex} + \text{puberty} + \text{puberty} * \text{sex} \\ \text{Amygdala volume} &= \text{intercept} + \text{age} + \text{sex} + \text{puberty}\end{aligned}$$

For all analyses, pairwise post-hoc contrasts comparing pre- and postpubertal males and females were investigated using the least-squares means procedure; corrected 2-tailed p values reported using the Tukey correction for multiple comparisons. As described below (see Results), a significant puberty by sex interaction was found in the bilateral hippocampus. In order to further investigate this effect, follow-up supplementary analyses display the effect of age split by sex when puberty is alternately accounted for or left unmodeled. Puberty by age interactions and 3-way interactions were investigated but found to be nonsignificant and not retained in the model. Nonlinear effects were investigated using quadratic regression but were not significant beyond linear effects and thus not pursued. All statistical analyses were conducted using R.⁴⁴

As described below (see Results), the above analysis revealed that hippocampal volumes differed between postpubertal males and females but not prepubertal males and females. In order to determine whether the observed postpubertal hippocampal volume differences were global or could be localized further within the hippocampus, we performed a vertex analysis that compared hippocampal shape in postpubertal males and females using the deformable mesh models generated by FIRST. Postpubertal males and females were compared using a linear regression that also included age as a covariate in the model. Results were considered significant if they surpassed a cluster-corrected threshold of $z > 2.33$, $p < 0.05$ as estimated using Gaussian random field theory.⁴⁵ Results of the vertex analysis were displayed using KWMeshVisu.⁴⁶

RESULTS

Sex Differences in the Effect of Puberty Upon Hippocampal Volume

We evaluated the effects of puberty in male and female subjects controlling for age and age by sex interactions. Notably, there was a significant puberty by sex interaction in both the left [$t(518)=2.3$; $p=0.02$] and the right hippocampus [$t(518)=2.4$; $p=0.02$], reflecting the fact that hippocampal volumes declined more in postpubertal males than females (Figure 2). Pairwise post-hoc testing demonstrated that the observed puberty by sex interaction was

driven by significant differences between males and females that were only seen after puberty. Whereas hippocampal volumes of prepubertal males and females were not significantly different [left: $t(518)=0.8$, $p=0.45$; right: $t(518)=1.1$, $p=0.27$], postpubertal females had significantly larger hippocampi than males bilaterally [left: $t(518)=4.5$, $p=1.0\times 10^{-5}$; right: $t(518)=4.2$, $p=3.0\times 10^{-5}$]. Furthermore, postpubertal males had smaller hippocampi than prepubertal males [left: $t(518)=3.7$, $p=5.5\times 10^{-4}$; right: $t(518)=3.9$, $p=1.2\times 10^{-4}$]; there was a similar nonsignificant trend in females [left: $t(518)=1.5$, $p=0.14$; right: $t(518)=1.9$, $p=0.06$]. Notably, an age by sex interaction that was present when puberty was not controlled for was absent [left: $t(518)=1.0$, $p=0.31$; right: $t(518)=1.3$, $p=0.21$] once the puberty by sex interaction was modeled (see Figure S1).

Even when accounting for puberty, there was a significant main effect of age, reflecting increased hippocampal volume with older age [left: $t(518)=2.0$, $p=0.05$; right: $t(518)=2.1$, $p=0.04$]. We also found that there was a trend towards a main effect of puberty, associated with diminished right hippocampus volume [$t(518)=1.9$, $p=0.06$]; the effect on the left was directionally similar [$t(518)=1.5$, $p=0.14$]. Taken together, these results demonstrate clear sex differences in the effect of puberty on the hippocampus, above and beyond general effects of age.

Postpubertal Sex Differences Are Most Prominent in Lateral Hippocampus

In order to determine whether postpubertal hippocampal volume differences between males and females were global or could be localized further within the hippocampus, we performed a vertex analysis examining hippocampal shape using the deformable mesh models generated by FIRST. As displayed in Figure 3, postpubertal sex differences in hippocampal morphology were seen on the lateral surface of the body of both the right and left hippocampus as well as the tail of the left hippocampus (with a similar sub-threshold effect in the right hippocampal tail). In these regions, females were found to have larger hippocampi than males. This region lies mainly within the lateral zone defined by Wang *et al.*, which approximates the CA1 subfield of the hippocampus.⁴⁷

Sex Differences in the Effect of Puberty Are Not Seen in the Amygdala

In contrast to the hippocampus, we did not find evidence for a puberty by sex interaction in the amygdala [right: $t(519)=0.39$, $p=0.70$; left: $t(519)=0.68$, $p=0.50$; Figure 4]. Once this nonsignificant interaction term was removed from the model, there was a main effect of age on the right amygdala reflecting increased volume with age [$t(520)=2.6$, $p=0.01$]; there was not a significant age effect on the left [$t(520)=0.8$, $p=0.41$]. There was also trend towards a main effect of sex was present on the left [females > males; $t(520)=1.8$, $p=0.06$]; volumes were equal between sexes on the right [$t(520)=0.1$, $p=0.91$]. On both the left and the right, there was a trend towards a main effect of puberty, with diminished amygdala volume following puberty, but this effect was not significant [right: $t(520)=1.9$, $p=0.06$; left: $t(520)=1.5$, $p=0.12$].

DISCUSSION

These findings, from the largest study to date regarding the evolving structure of the hippocampus and amygdala during adolescence, demonstrate that emerging sex differences in hippocampal volume are in part due to specific influence of puberty. While hippocampal volumes were similar in prepubertal males and females, following puberty females had significantly larger hippocampi than males. A vertex analysis demonstrated that differences between postpubertal males and females are most prominent in the lateral aspect of the hippocampus bilaterally, corresponding to the CA 1 subfield. These results were specific to the hippocampus, as there was no significant differential effect of puberty on the amygdala.

Sex differences in the medial temporal lobe structure were established by Giedd *et al.* (1996). We replicate these results in bilateral hippocampus, and show that hippocampal volumes increase with age in females but decline in males. However, studies that have examined the degree to which such sex differences might be due to puberty have produced relatively heterogeneous results.⁴⁸ In a sample of 54 girls, Blanton *et al.* (2012) found that puberty was associated with diminished hippocampus volume;⁴⁹ however, no males were available for comparison in that study. Similarly, Neufang *et al.* (2009) found in a sample of 46 subjects that puberty was associated with diminished hippocampus volumes in both boys and girls.³⁶ Notably, however, neither study controlled for effects of age in the analysis of puberty. In contrast, a recent study by Bramen *et al.*, 2011 explicitly attempted to dissociate these 2 collinear effects in a sample of 80 subjects.³⁷ As in Blanton *et al.* and Neufang *et al.*, Bramen *et al.* found that puberty was associated with declining hippocampal volumes in females. In contrast, they reported that puberty was associated with larger volumes in postpubertal males. Finally, while this manuscript was in preparation, Hu *et al.* (2013) reported in a sample of 306 subjects that pubertal stage is positively associated with hippocampal volume in girls, but that a negative relationship exists in boys even while controlling for age.⁵⁰ It should be noted that several other studies have examined the effect of puberty, sex hormones, or polymorphisms of the androgen receptor and either did not find significant effects in the medial temporal lobe⁵¹ or did not evaluate it due to a focus on cortical thickness.^{9,52}

Taken together, while one study (Bramen *et al.*, 2011) reported that puberty is associated with enlargement of the hippocampus in boys, the bulk of the prior evidence including one large (Hu *et al.*, 2013) and one small study (Neufang *et al.*, 2009) suggests the opposite. Our data supports this conclusion, with postpubertal boys having significantly smaller hippocampi bilaterally, even while accounting for age. The overall picture for girls is more mixed: 3 prior studies^{36,37,49} have reported that puberty in females is associated with a decline in hippocampal volume, whereas the large recent study by Hu *et al.* found the opposite result.

Despite the power afforded by our large sample size, we did not find a significant effect of puberty on the hippocampal volumes of females. In contrast to the clear effects of puberty on the hippocampus in males, this suggests that any such effects in females are quite subtle to the degree that they are present at all. It should be noted, however, that when puberty is not accounted for, hippocampal volumes on average increase with age in females but decline with age in males (replicating prior results reported by Giedd *et al.*, 1996). In our data, this age effect in females was not specifically linked to puberty, while the volume loss with age in males was attributable to puberty. When controlling for the specific effects of puberty, there was no difference in the observed patterns of development in males and females. Although speculative, this may be due to rising estrogen levels in pubertal females helping to preserve relative hippocampal volume over time, preventing the volume loss seen in males with puberty.

While these volumetric results clarify the existing literature, the results of the vertex analysis provide novel data localizing the effects of puberty. Specifically, the vertex analysis demonstrates that postpubertal differences in hippocampal shape between males and females are found mainly in the lateral aspect of the hippocampus. This region which lies mainly within the lateral zone defined by Wang *et al.* (2006), and corresponds to the CA1 subfield where animal models have demonstrated the impact of estrogens.⁴⁷ Using a longitudinal design, Gogtay *et al.* (2006) found that the volume of a similar region of the hippocampus including the tail is relatively constant over the adolescent period, whereas the hippocampal head declines in volume.⁵³ However, because this study was too small to separately consider sex or pubertal status, important heterogeneity in trajectories of hippocampal development

may not have been apparent. Neufang *et al.* (2009) similarly found that females had larger posterior hippocampus volumes than males. Our results indicate that the changes during adolescence in the lateral and posterior hippocampus are sex- and puberty- dependent. These findings may be relevant for a large variety of processes that undergo rapid evolution during adolescence and exhibit known sex differences, including cognition, decision making, and risk-taking behaviors.⁵⁴ For example, we have recently demonstrated in a very large sample of adolescents (n=3,500) that female adolescents perform better in several cognitive domains including attention, episodic memory, and tests of social cognition such as emotion identification.⁵⁵ As the hippocampus has been implicated in many of these processes,⁵⁶ understanding how puberty-related changes in hippocampal morphometry relate to sex differences in cognition is an important area for future research.

Notably, the results presented here find some interesting parallels in prior studies from animal models.¹⁷ Estrogens preserve CA1 structure in rats, and lack of estrogens leads to a profound loss in dendritic spines of CA1 pyramidal cells.¹⁹ Estrogens also have been shown to be critical for preserving axonal sprouting in the hippocampus,²⁰ and have been linked to neurogenesis in the MTL.^{21,22} This accords with prior data from Neufang *et al.*, (2009), who reported a positive correlation between estrogen levels and parahippocampal gray matter density,³⁶ suggesting that rising estrogen levels in pubertal females may help preserve hippocampal volume in females, especially in comparison to males.

In contrast to the clear sex differences in the effects of puberty on the hippocampus, we did not find a sex-specific effect of puberty on the amygdala. Instead, we found that puberty was associated with a non-significant trend towards amygdala volume reduction bilaterally in both sexes. However, age effects in the right amygdala were also present, resulting in a reduction in left > right laterality during adolescent development. Prior studies have generally not reported significant effects of age in the right amygdala,^{36,37,49–51}, although the original study by Giedd *et al.* (1996) did find an increase in left amygdala volume (in males only). We suspect that the results seen here were not detected by prior studies mainly due to greatly increased power of our larger sample, perhaps in combination with differences in analytic approach.

While this study capitalizes upon a large sample size and applies a vertexwise analyses, several limitations should be noted. First, all data presented here is cross-sectional; further description of developmental trajectories requires longitudinal data.⁵⁷ Follow-up studies will track brain development on a longitudinal basis, allowing tighter links to be established between pubescence, hippocampal morphometry, and neurocognition. Second, mechanistic interpretation is limited by the lack of information regarding circulating hormone levels in participants. Therefore, while prior imaging literature and animal models suggest roles for gonadal hormones in the development of MTL morphometry, the presence of any such link in the present data must remain speculative. Third, while this study utilized a previously validated self-report measure of pubertal assessment, such measures are known to be less accurate than a physician examination. Fourth, while the vertex analyses of the current results provide substantial additional detail beyond summary measures such as region volume, the analysis method utilized (FSL's FIRST utility) does not allow precise delineation of hippocampal subfields. Further investigation of these results using ultra-high resolution MRI and dedicated hippocampal subfield segmentation⁵⁸ will be useful in future studies. Finally, it should be noted that while our results were significant, statistical power was limited by the unequal sizes of the prepubertal and postpubertal groups.

These limitations notwithstanding, the results establish that puberty has a different effect on hippocampal structure in males and females, suggesting that hormonal mechanisms impacting hippocampal structure in animal models are likewise important in humans. Our

findings encourage investigation into the potential role of these interactions in psychiatric disorders with adolescent onset and strong gender disparities, such as depression, anxiety disorders, and schizophrenia.^{28,29}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Segmentation of the right hippocampus in a single subject performed using FSL's FIRST procedure.

Figure 2.

Sex differences in the impact of puberty on hippocampal volume. Note: Prepubertal males and females have similar hippocampal volumes. However, postpubertal males have significantly reduced hippocampal volume compared to postpubertal females. Error bars indicate standard error of the mean, reported hippocampal volumes control for intracranial volume (ICV), subject age, and an age by sex interaction.

Figure 3.

Lateral views of hippocampal vertex analyses, demonstrating regional differences in hippocampus morphology in postpubertal males and females. Note: In both right (**A**) and left (**B**) hippocampus, males were found to have reduced hippocampal volume on the lateral aspect of the hippocampal body (red, indicating cluster-corrected $z > 2.33$, $p < 0.05$). Hippocampal mesh models displayed using KWMeshVisu. Analyses control for intracranial volume, subject age, and an age by sex interaction.

Figure 4.

Sex-specific effects of puberty on the hippocampus are regionally specific and not present in the amygdala. Note: A nonsignificant trend for a main effect of puberty was observed bilaterally, as well as a trend towards larger left amygdala volumes in females. Error bars indicate standard error of the mean, reported hippocampal volumes control for intracranial volume (ICV) and subject age.

Table 1

Sample Demographics

Sample	n	Mean age (y)	Mean Tanner	# Right Handed
<i>Prepubertal</i>				
Males	57	11.6	1.6	49
Females	49	12.6	1.6	35
<i>Postpubertal</i>				
Males	132	18.2	5	118
Females	286	16.9	5	245