Increases in Sex Hormones during Anti-Tumor Necrosis Factor α Therapy in Adolescents with Crohn's Disease

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Objective To evaluate children with Crohn's disease for inverse relationships between systemic inflammatory cytokines and sex hormone regulation in the context of anti-tumor necrosis factor α (TNF-α) therapy.

Study design An observational study design was used to assess sex hormone and gonadotropin levels at the time of initiation of anti-TNF-α therapy and 10 weeks and 12 months later in 72 adolescents (Tanner stage 2-5) with Crohn's disease. Mixed-model linear regression was used to evaluate relationships between hormone levels, systemic inflammation, and dual-energy x-ray absorptiometry whole-body fat mass Z scores over the study interval.

Results Sex hormone Z scores increased significantly during the 10-week induction interval: testosterone Z scores in male patients increased from a median of −0.36 to 0.40 ($P < .05$) and estradiol Z scores in females increased from −0.35 to −0.02 ($P < .01$). In mixed model regression, the pediatric Crohn's disease activity index score, cytokine levels, and measures of inflammation were significantly and negatively associated with sex hormone Z scores and with luteinizing hormone and follicle-stimulating hormone levels, adjusted for sex and Tanner stage. Sex hormone and gonadotropin levels were not associated with body mass index or fat mass Z-scores.

Conclusions Crohn's disease is associated with delayed maturation, and initiation of anti-TNF-α therapy was associated with significant and rapid increases in sex hormone and gonadotropin levels, in association with improvements in disease activity and measures of inflammation. These data are consistent with preclinical studies of the effects of inflammation on sex hormone regulation. *(J Pediatr 2016;171:146-52)*.

Chronic inflammatory diseases such as Crohn's disease are associated with delays in the onset and progression of puberty,[1,2] with potential sequelae including poor linear growth,[3,4] impaired bone accrual,[5,6] and decreased quality of life.[7,8] Studies in animals have demonstrated that the introduction of inflammatory cytokines and other inflammatory mediators resulted in decreased release of luteinizing hormone (LH) from the pituitary gland.[9] Furthermore, induction of colitis resulted in lower testosterone and estradiol levels compared with pair-fed controls,[10-12] with levels of gonadotropins that did not demonstrate the normal feedback increase in response to low levels of sex hormones. As such, the suppression of sex hormones in animal models of inflammation appeared to be centrally mediated and thus hypogonadotropic in nature. In addition, mice with colitis had longer delay of puberty than seen in mice who were food restricted, suggesting that this delay was caused by factors besides body fat and levels of the adipokine leptin—both known to play a permissive role in sex hormone production.[10-14] In mice with experimental colitis, treatment with a monoclonal antibody against tumor necrosis factor α (TNF-α) resulted in partial normalization of estrogen production, as evidenced by earlier vaginal opening, an estrogen-dependent marker of pubertal progression in murine models.[15]

Studies of children and adolescents with Crohn's disease have reported delays in bone age,[16,17] breast development,[18] menarche,[17-19] testicular enlargement,[18] and the pubertal growth spurt[16,20]—delays that persisted despite improvements in disease treatment.[2] Previous studies of the impact of Crohn's disease therapy on sex hormone levels in adolescents with Crohn's disease are limited to a case report in a male and a series in adolescents enrolled at a highly variable interval after starting anti-TNF-α therapy.[7,21]

BMI	Body mass index
CRP	C-reactive protein
CV	Coefficient of variation
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
LH	Luteinizing hormone
PCDAI	Pediatric Crohn's Disease Activity Index
TNF-α	Tumor necrosis factor α

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Therefore, the regulation of sex hormones in pubertal children with Crohn's disease remains understudied.

This study assessed changes in sex hormone levels and biomarkers of inflammation in a cohort of pubertal and postpubertal adolescents with Crohn's disease after initiation of treatment with infliximab, a monoclonal antibody against TNF-α. Our hypothesis was that before anti-TNF-α treatment, male and female adolescents have testosterone and estradiol levels below the reference ranges, respectively, and without compensatory increases in gonadotropin levels. We further hypothesized that sex hormone levels would increase after treatment with anti-TNF-α treatment, and reductions in measures of inflammation (more so than increases in measures of body fat) would be associated with greater increases in sex hormones. These data may offer insights into the central regulation of sex hormone production and pubertal progression in the setting of chronic inflammatory disease.

Methods

This study is ancillary to a previous prospective cohort study of bone and mineral metabolism in 90 children and adolescents, ages 5-21 years, enrolled at the time of initiation of anti-TNF-α therapy at the Children's Hospital of Philadelphia.[22,23] Participants were excluded for previous anti-TNF-α therapy or medical illness or therapies unrelated to Crohn's disease that could potentially affect bone, nutrition, or growth. Study visits were completed at the time of the first anti-TNF-α infusion (baseline visit), and 10 weeks, 6 months, and 12 months later. The study protocol was approved by the Children's Hospital of Philadelphia Institutional Review Board. Informed consent was obtained from participants $\geq$18 years of age, and a parent or guardian of participants <18 years. Assent was obtained from participants 7-18 years of age. The informed consent covered the ancillary measures described here.

Short–term changes in mineral metabolism have been reported, demonstrating significant increases in parathyroid hormone and $1,25(OH)_2$ vitamin D levels in association with improvements in disease activity[22] and decreases in cytokine and C-reactive protein (CRP) levels. Improvements in disease activity over the first 10 weeks were associated with gains in height, trabecular bone mineral density, and cortical structure during the 12-month study interval.[23] This study of sex hormones and maturation is limited to participants who were Tanner stages 2 through 5 at enrollment. Of the 76 who were eligible according to Tanner stage, 72 had at least 1 specimen available for ancillary measures.

Disease characteristics, medications, and anthropometry were recorded at each visit. Height was measured with a stadiometer and weight with a digital scale. Height and body mass index (BMI, kg/m^2) were converted to sex-specific Z scores relative to age by the use of national growth charts.[24] Tanner stage was ascertained by self-assessment questionnaire at baseline and 12-month visits.[25] For the 10-week visit, participants were classified as being at the same Tanner stage as at baseline. Females were asked if they had started their menstrual period; however, the regularity of menses was not assessed. Disease activity was assessed using the Pediatric Crohn's Disease Activity Index (PCDAI) based on symptoms (30%), physical examination (30%), laboratory variables (20%), and growth (20%), with scores ranging from 0 to 100.[26] Disease activity was categorized as none (1-10), mild (11-30), and moderate to severe (>30).

At the baseline and 12-month visit, whole-body dual-energy x-ray absorptiometry scans were obtained with a Hologic Delphi densitometer (Bedford, Massachusetts) with a fan beam in the array mode. The results for whole body fat mass, excluding the head, were converted to sex- and race-specific Z scores relative to age, based on our 921 healthy reference children and adolescents, as previously described.[6] Referent children also performed self-assessment of Tanner stage as described previously.[25]

Laboratory studies at each visit included hematocrit, erythrocyte sedimentation rate (mm/h), and serum CRP (mg/L), and albumin (g/dL) concentrations, analyzed by the use of standard methods in the clinical laboratory. Serum TNF-α and interleukin-6 were measured via the Human Cytokine Six-Plex High-Sensitivity Antibody Bead Kit on a Luminex instrument (Millipore, Billerica, Massachusetts) with a sensitivity of 0.08 and 0.10 pg/mL and interassay coefficient of variation (CV) of 8.3% and 7%, respectively.[22]

Testosterone and estradiol were tested at Quest Diagnostics (Madison, New Jersey) by the use of an ultrasensitive liquid chromatography-tandem mass spectrometry assay with sensitivity down to 1 ng/dL for testosterone and 2 pg/mL for estradiol. For testosterone, the intraassay CVs were 7.1 and 10.5 and the interassay CVs were 7.6 and 10.8 at testosterone levels of 9.6 and 43.7 ng/mL, respectively. For estradiol, the intraassay CVs were 15.3 and 10.4 and the interassay CVs were 7.7%-15.3% and 9.9%-14.0% at estradiol levels of 10 and 200 pg/mL. Testosterone and estradiol values were converted to Z scores on the basis of the mean and SD of reference values from Quest (**Table I**; available at www.jpeds.com). For cases in which the lower limit of 2 SDs below the mean dropped below 0, Z score was calculated as Z score = [LOG10(measured value) − LOG10(reference mean)]/(reference SD/reference mean).[27] For testosterone, the Z scores were Tanner-stage-specific. For females, the estradiol reference data were available according to age range (1-9, 10-11, 12-14, and 15-17 years). Because of the high proportion with delayed maturation (55% of females), Z scores were generated with the use of bone age, including the baseline bone age for baseline and 10-week estradiol levels and the 1-year bone age for the 1-year estradiol levels. LH and follicle-stimulating hormone (FSH) were analyzed at the University of Virginia Center for Research in Reproduction Ligand Core Laboratory via chemiluminescence. Manufacturer, assay sensitivity, and intra- and interassay CVs for LH and FSH measurements had sensitivities of 0.1 and 0.05 IU/L,