Sex-Specific Pathways Lead to Statural Growth Impairment in Children with Crohn's Disease

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Objectives To examine the underlying mechanisms that lead growth impairment to occur more commonly in males than females with Crohn's disease (CD).

Study design Children and adolescents with CD were enrolled in a prospective multicenter longitudinal cohort study. Height Z-score difference was computed as height Z-score based on chronological age (height chronological age-Z-score) minus height Z-score based on bone age (height bone age-Z-score) using longitudinal data. Specific serum cytokines were measured, hormone Z-scores were calculated based on bone age (bone age-Z), and their longitudinal associations were examined.

Results There were 122 children with CD (63% male) who completed 594 visits. The mean ± SD chronological age was 11.70 ± 1.79 years. The mean ± SD height chronological age-Z-score was −0.03 ± 0.99 in males and −0.49 ± 0.87 in females. The mean ± SD height bone age-Z-score was 0.23 ± 0.93 in males and 0.37 ± 0.96 in females. The magnitude of the mean height Z-score difference was greater in females (−0.87 ± 0.94) than males (−0.27 ± 0.90; $P = .005$), indicating growth was better in females than males. The following negative associations were identified: in females, interleukin (IL)-8 ($P < .001$) and IL-12p70 ($P = .035$) with gonadotropin-bone age-Z-scores; IL-8 ($P = .010$), IL-12p70 ($P = .020$), and interferon-γ ($P = .004$) with sex hormone-bone age-Z-scores, and IL-8 ($P = .044$) and interferon-γ ($P < .001$) with insulin-like growth factor 1-bone age-Z-scores; in males, IL-1 beta ($P = .019$) and IL-6 ($P = .025$) with insulin-like growth factor 1-bone age-Z-scores.

Conclusions Our data suggest that sex-specific molecular pathways lead to growth impairment in children with CD (primarily growth hormone/insulin-like growth factor-1 axis in males and primarily hypothalamic-pituitary-gonadal axis in females). Mapping these sex-specific molecular pathways may help in the development of sex-specific treatment approaches targeting the underlying inflammation characteristic of CD. *(J Pediatr 2022;249:75-83)*.

Statural growth is a dynamic marker of disease activity/disease severity in children with Crohn's disease (CD). Growth impairment, both a marker for and complication of poorly controlled CD, occurs in up to 80% of patients,[1-19] more commonly in males than females.[1-6,8,11-16] The impact of CD on growth is mediated by many factors, including inflammation, genetics, the microbiome, nutrition, and medications. The prevalence of growth impairment in CD has not changed in more than 25 years.[1-19] Normalization of growth is primarily a marker of disease control and successful therapy, and the continued presence of growth impairment primarily reflects continued suboptimal treatment of the underlying inflammation characteristic of CD.[1-19]

The Growth Study is a prospective multicenter longitudinal cohort study examining sex differences in growth impairment in children with CD. We first

BMI	Body mass index
CD	Crohn's disease
FSH	Follicle-stimulating hormone
HPG	Hypothalamic-pituitary-gonadal
IBD	Inflammatory bowel disease
IFN	Interferon
IGF-1	Insulin-like growth factor 1
IL	Interleukin
LH	Luteinizing hormone
TNF	Tumor necrosis factor
wPCDAI	Weighted pediatric Crohn's disease activity index

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demonstrated that sex differences in growth persist in a contemporary cohort of children with CD despite treatment with current medications.[1] We next identified several clinical variables (ie, disease characteristics, symptoms/extraintestinal manifestations, and medications) associated with sex-specific growth in children with CD.[2] Each of the identified variables was more strongly associated with statural growth in one sex than the other. Treatment with biologics, methotrexate or vitamin D was associated with better growth in males, and treatment with azathioprine/6-mercaptopurine or probiotics was associated with worse growth in females.[2] These findings suggest the presence of sex-specific molecular pathways leading to growth impairment in CD, and a sex difference in treatment response.[2]

Our central hypothesis is that the inflammation characteristic of CD has worse effects on endocrine growth regulators (hormones) in males.[1,2,4-6] The growth hormone/insulin-like growth factor 1 (IGF-1) axis and hypothalamic-pituitary-gonadal (HPG) axis are the major endocrine regulators of growth. IGF-1 is the primary mediator of growth hormone-stimulated growth. Estradiol is the primary hormone involved in skeletal maturation and the pubertal growth spurt in both sexes.[20,21]

In a previous cross-sectional study, we found that IGF-1 Z-scores were lower in males than females with pediatric CD. Nonspecific inflammatory markers such as erythrocyte sedimentation rate, C-reactive protein, and albumin levels predicted IGF-1 Z-scores but did not differ by sex and were associated with testosterone and luteinizing hormone (LH) Z-scores in males but not with estradiol and follicle-stimulating hormone (FSH) Z-scores in females.[4] Decreased androgen levels have been observed in males with other chronic inflammatory conditions.[22-24] In vitro models suggest that inflammatory cytokines (eg, tumor necrosis factor [TNF]-alpha) decrease testosterone.[25] Several investigators have implicated inflammatory mediators in impacting the growth hormone-IGF-1-statural growth pathway at various points.[26-28] Multiple correlations have been reported between IGF-1 and inflammatory markers in CD, as in our cross-sectional study.[4,29,30] These findings suggest inflammation exerts a greater negative impact on hormone levels and statural growth in males with CD.[4,6] Inflammation seems to exert a worse effect on hormone levels and growth in males.[1,2,4-6,22-30]

Our aims were to analyze prospectively collected longitudinal data to compare height Z-score difference (height Z-scores calculated based on chronological age [height chronological age-Z-scores] minus height Z-scores calculated based on bone age [height bone age-Z-scores]) by sex and examine the associations between 10 specific cytokines and 5 specific hormones by sex.[1,2] We theorized that the specific cytokines associated with specific hormones will differ by sex, suggesting that sex-specific molecular pathways lead to growth impairment in CD. We also conducted a mediation analyses to provide additional evidence for sex-specific molecular pathways if found.

Methods

There were 205 children and adolescents with a final diagnosis of CD (females with chronological age of ≥5 years and <14 years; males with chronological age of ≥6 years and <16 years) who participated in a screening visit at one of eight medical centers between April 2015 and April 2019 to determine eligibility for study visit participation in the Growth Study as previously described.[1,2] Study visit 1 is targeted to occur 3 months after the screening visit and 1 study visit is targeted to occur every 6 months for a total of 5 study visits (ie, a projected 27-month follow-up period from the time of the screening visit). There were 122 participants with CD who completed the screening visit and more than 1 study visit (594 visits). Enrollment numbers were planned based on our power calculation before initiating this study. We aimed to detect small to moderate standardized effects (0.39-0.57) with adequate power (≥80%) in a 2-sided .05-level test when evaluating sex differences in height Z-score differences and the association of specific cytokines with specific hormones. When our target enrollment was reached, we stopped screening potential participants.

Bone age assessed by left hand radiograph allows for clinically meaningful interpretation of growth and growth potential in CD.[1,2,31-33] During the screening visit, the participant was eligible to undergo bone age assessment if the participant met the following previously described specific criteria: (1) CD diagnosed by standard criteria; (2) Tanner stage 1-4; (3) no prior bone age result exceeding study eligibility criteria (bone age of >12 years for females or >14 years for males exceeds eligibility criteria to capture children with ≥3 bone age years of growth potential remaining, allowing adequate study of height velocity during the 2-year follow-up period); (4) not having completed statural growth as assessed by the standard of care pediatric gastroenterologist; (5) no liver disease; (6) no other known cause of growth delay; (7) no other poorly controlled medical condition; (8) no history of pregnancy; (9) no history of treatment with growth hormone, testosterone, or estrogen; and (10) available for follow-up for the duration of the study period.[1,2] If a patient met these specific criteria and had not been exposed to corticosteroids (intravenous, oral, intranasal, inhaled, rectal, topical, eye drops, swish-and-spit/oral rinse) in the 56 days[34] preceding the screening visit (because recent use suppresses the growth hormone axis), a bone age was obtained.

Females with a bone age of 4 years 2 months or greater and 12 years or less and males with a bone age of 5 years or greater and 14 years or less qualified to participate in study visits. Puberty is of longer duration in males, explaining the broader chronological age and bone age range for eligibility criteria in males. Study visits were conducted if the participant continued to meet the eligibility criteria.

Bone age, obtained at the screening visit and at up to 2 study visits, was blindly interpreted by 1 investigator using the standards of Greulich and Pyle as previously

described.[1,2,4,31,32,35] Because bone age reference values vary by sex and chronological age, bone age results were transformed into bone age Z-scores using standard reference values for all females with a chronological age of 15 or less and males with a chronological age of 17 years or less, because the epiphyses close at a bone age of 15 years in females and a bone age of 17 years in males.[1,2,31,33,36]

Comprehensive clinical information, anthropometric measurements, and laboratory tests were collected from each participant at each study visit.[1,2] It is beyond the scope of this report to provide details of the extensive information collected. We provide a description of variables included in this report.

The variables race, Asian, ethnicity, Tanner stage, chronological age, bone age, date of initial inflammatory bowel disease (IBD) diagnosis, initial classification of IBD, date of CD diagnosis, and disease duration were defined previously.[1,2] Reported use of medications of interest included biologics (adalimumab, certolizumab, infliximab, natalizumab, ustekinumab, and vedolizumab), immunomodulators (azathioprine/6 mercaptopurine, methotrexate, cyclosporine, and tacrolimus), and corticosteroids.[2] The weighted pediatric CD Activity Index (wPCDAI) is a measure of disease activity in children with CD.[37]

Laboratory measures analyzed for each participant (each male and each female) at each study visit included the erythrocyte sedimentation rate, C-reactive protein, albumin (at local laboratories), IGF-1, testosterone, estradiol, LH, FSH (Esoterix Endocrinology), and 10 specific cytokines: interleukin (IL)-1 beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, interferon (IFN)-γ, and TNF-α. Serum concentrations of cytokines were determined in the Weill Cornell Medicine CTSC Core Laboratory using a quantitative electrochemiluminescence-based assay (K15049G) and the MESO QuickPlex SQ120 Analyzer from Meso Scale Discovery following the manufacturer's instructions. Assays were run in duplicate with quality controls. Intra-assay coefficients of variation for these cytokines range from 2.4% to 7.2%, and interassay coefficients of variation range from 5.0% to 10.5%. Detection limits of IFN-γ, IL-1*B*, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α are 0.40 pg/mL, 0.10 pg/mL, 0.10 pg/mL, 0.016 pg/mL, 0.10 pg/mL, 0.03 pg/mL, 0.077 pg/mL, 0.10 pg/mL, 0.50 pg/mL, and 0.20 pg/mL, respectively. All cytokine data were log-transformed for statistical analyses.

Hormone results were converted to Z-scores based on reference values. IGF-1 Z-scores were determined using means and SDs specific to age and sex provided by Esoterix Endocrinology. The SDs for gonadotropins and sex hormones were computed from the mean and upper and lower bounds of the normal ranges, accounting for asymmetry about the mean if present. For gonadotropins and sex hormones, the means and ranges used to calculate Z-scores were specific to sex, age, and Tanner stage. Because pubertal growth acceleration correlates more closely with bone age than with chronological age,[32,38] we calculated hormone Z-scores based on bone age (ie, hormone bone age-Z-scores).[4]

Because serum testosterone and estradiol are markers of pubertal status in males and females, respectively, we assessed sex hormone levels using a composite measure defined as testosterone bone age-Z-score in males and estradiol bone age-Z-score in females.[4] Because LH is the primary regulator of testosterone synthesis in Leydig cells, and FSH is the primary regulator of estradiol synthesis in granulosa cells, via stimulation of aromatase (aromatase is an enzyme responsible for a key step in the biosynthesis of estradiol from testosterone[39]), we assessed gonadotropin levels using a composite measure defined as LH bone age-Z-score in males and FSH bone age-Z-score in females.[4]

Weight and height were measured using a digital scale to the nearest 0.1 kg and stadiometer to the nearest 0.1 cm, respectively; body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. The Z-scores were calculated based on chronological age and bone age for weight, height, and BMI using reference tables from the Centers for Disease Control and Prevention, National Center for Health Statistics.[40] The height, weight, or BMI Z-score difference was calculated as variable chronological age-Z-score minus variable bone age-Z-score.[1,2] Height velocities were calculated as the change in height during each interval between study visits, divided by the interval time between study visits, and then standardized based on bone age using age- and sex-specific references.[41]

Statistical Analyses

Descriptive statistics were generated for demographic characteristics and key variables of interest measured at the time of the screening visit. These variables were compared between males and females using the *t* test for continuous variables and the χ^2 test for categorical variables. Longitudinal data were collected throughout the study period from 122 participants with a total of 594 observations (visits) available for analysis. We used a generalized linear model with identity link function to assess the sex difference for each outcome of interest. A generalized estimating equation was used to account for within-subject correlation owing to repeated measures from the same participant. Owing to the high correlation between Tanner stage and bone age (correlation coefficient = 0.692; $P < .001$), we use bone age in our calculations as a more quantitative measure directly relevant to skeletal growth. When assessing the sex difference of bone age Z-score, height velocity bone age-Z-score, and anthropometric Z-score differences, the analysis model also included biologic treatment, immunomodulator treatment, and the wPCDAI to adjust for potential confounding. We report the unadjusted mean, SD, and range for each outcome by sex and adjusted mean sex difference, its corresponding 95% CI, and *P* values. In addition, we also examine the association between cytokines and hormone bone age-Z-scores by sex using a similar analytic approach (generalized linear model with generalized estimating equation) and report the regression coefficient corresponding with the specific cytokine to represent its strength of association with the specific hormone bone age-Z-score. We also evaluated whether the

strength of each of these associations differed by sex. For these analyses (association between specific cytokine and specific hormone bone age-Z-score conducted for each sex), the model includes the specific cytokine and potential confounding factors (ie, biologic treatment, immunomodulator treatment, wPCDAI, and chronological age). The model that examines the sex difference (of the strength of association between a specific cytokine and specific hormone bone age-Z-score in males vs females) includes sex and the specific cytokine-by-sex interaction in addition to the variables listed above. For each of the sex-specific analyses, we show the regression coefficient of the specific cytokine; when comparing the sex difference in strength of association between the specific cytokine and specific hormone bone age-Z-score, we report the regression coefficient corresponding with the cytokine-by-sex interaction. Finally, we conducted exploratory analyses to examine whether each specific hormone mediates the effect of each specific cytokine on height velocity (adjusting for potential confounding factors; ie, biologic treatment, immunomodulator treatment, wPCDAI, and chronological age). A mediation analysis was conducted using the test of joint significance involving a potential mediator. A specific hormone is declared a mediator if and only if both the test of the regression coefficient of a specific cytokine on a specific hormone and the test of the coefficient of that specific hormone on height velocity controlling for that specific cytokine are both significant at an alpha level of 0.05, 2 tailed.[42] This approach showed its optimality when compared with 13 other methods for mediation analysis and allows for partial mediation which we expect to occur (as opposed to total mediation). For each of the reported regression coefficients, we also present its corresponding 95% CI and P value. Findings with 2-sided P values of .05 or less are declared as statistically significant. Because of the hypotheses-generating nature of these analyses, we did not apply multiple comparison adjustment to the P values to ensure sufficient power to identify clinically meaningful associations for future investigation.[43] SIR/XS version 19 (ASIR Database Software, Terrey Hills, Australia) relational database management system software was used to capture and store the data; CITRIX (CITRIX System, Fort Lauderdale, FL) application-server was used to securely enter and transmit the data over the internet. Data were analyzed using SPSS Version 27 (SPSS, Inc).

Study Approval

Each participating center obtained institutional review board approval for the study protocol, and written informed consent and assent were obtained from parents or guardians and children (participants) before participation in the screening visit.

Results

There were 122 children with CD (63% male) who completed 1 or more study visits (**Table I**). Tanner stages 1 and 2 were more common in females at the time of the screening visit. Males had a slightly larger mean number of visits completed (screening visits + study visits) (male, 5.08 ± 1.29; range, 2-6); (female, 4.38 ± 1.50; range, 2-6; P = .010).

The mean chronological age at the time of the screening visit for females (10.76 ± 1.65 years; range, 6.19-13.36 years) was lower than for males (12.26 ± 1.64 years; range, 8.50-15.73 years; P < .001). The average disease duration since initial diagnosis of IBD was 3.54 ± 2.12 years (range, 0.22-10.23 years) in males and 2.77 ± 1.78 years (range, 0.28-8.13 years) in females (P = .032). The average disease duration since initial diagnosis of CD was 3.50 ± 2.11 years (range, 0.20-10.23 years) in males and 2.55 ± 1.65 years (range, 0.52-8.06 years) in females (P = .004). These sex differences are due to our sex-specific chronological age eligibility criteria.[1,2]

Longitudinal Data

The mean erythrocyte sedimentation rate was 12.45 ± 9.91 (range, 0.00-69.00) in males and 12.24 ± 8.12 (range, 0.00-52.00) in females (P = .873); the mean C-reactive protein was 2.44 ± 4.85 (range, 0.10-60.00) in males and 2.64 ± 3.49 (range, 0.04-22.60) in females (P = .754); the mean albumin was 4.10 ± 0.38 (range, 2.60-5.00) in males and 4.17 ± 0.40 (range, 3.30-5.20) in females (P = .315).

The low mean wPCDAI in males (5.36 ± 9.21; range, 0.00-42.00) and in females (7.07 ± 9.88; range, 0.00-44.50; P = .152) indicated remission.

Specific serum cytokine levels did not differ by sex (IFN-γ [P = .464], IL-1B [P = .459], IL-2 [P = .182], IL-4 [P = .689], IL-6 [P = .604], IL-8 [P = .351], IL-10 [P = .111], IL-12p70 [P = .983], IL-13 [P = .533] and TNF-α [P = .104]).

Overall, 58%, 44%, and 6% of females and 77%, 53%, and 5% of males were treated with biologics (P = .048), immunomodulators (P = .453), and corticosteroids (P = .999), respectively.

Bone Age, Height Velocity and Anthropometric Z-score Differences. The mean bone age Z-score was lower in females (P < .001). Height velocity bone age-Z-score did not differ by sex (P = .390) (**Figure 1**; available at www.jpeds.com). The mean ± SD height chronological age-Z-score was −0.03 ± 0.99 in males and −0.49 ± 0.87 in females. The mean ± SD height bone age-Z-score was 0.23 ± 0.93 in males and 0.37 ± 0.96 in females. The mean ± SD weight chronological age-Z-score was 0.10 ± 1.07 in males and −0.32 ± 1.09 in females. The mean weight bone age-Z-score was 0.29 ± 0.95 in males and 0.30 ± 0.83 in females. The mean BMI chronological age-Z-score was 0.13 ± 0.98 in males and −0.08 ± 1.09 in females. The mean BMI bone age Z-score was 0.22 ± 0.93 in males and 0.18 ± 0.94 in females (**Table II**). The magnitude of the mean height Z-score difference (P = .005) (**Figure 1**), weight Z-score difference (P = .004), and BMI Z-score difference (P = .006) was higher in females.

Table I. Demographics, Tanner stage, and baseline disease characteristics of participants (all 122 participants have a final diagnosis of CD)

Demographics and Tanner stage	Total N, (% of 122)	Female N, (% of 45)	Male N, (% of 77)	*P* value*
Race*				.922
Asian	5 (4.1)	1 (2.2)	4 (5.2)	
East Asian	2 (1.6)	2 (4.4)	0 (0)	
South Asian	4 (3.3)	0 (0)	4 (5.2)	
Black/African American	11 (9.0)	4 (8.9)	7 (9.1)	
Other	2 (1.6)	1 (2.2)	1 (1.3)	
White	102 (83.6)	38 (84.4)	64 (83.1)	
Declined	2 (1.6)	1 (2.2)	1 (1.3)	
Ethnicity*				.495
Hispanic or Latino	6 (4.9)	3 (6.7)	3 (3.9)	
Not Hispanic or Latino	116 (95.1)	42 (93.3)	74 (96.1)	
Tanner stage*				.019
1	45 (36.9)	21 (46.7)	24 (31.2)	
2	45 (36.9)	19 (42.2)	26 (33.8)	
3	23 (18.9)	5 (11.1)	18 (23.4)	
4	9 (7.4)	0 (0)	9 (11.7)	
Baseline disease characteristics				
Initial classification of IBD*†				.054
Ulcerative colitis	3 (2.5)	1 (2.2)	2 (2.6)	
Inflammatory bowel disease unclassified	6 (4.9)	5 (11.1)	1 (1.3)	
CD	113 (92.6)	39 (86.7)	74 (96.1)	
Upper Disease Location at Diagnosis*				.678
L4a: upper disease proximal to Ligament of Treitz	37 (30.3)	16 (35.6)	21 (27.3)	
L4b: upper disease distal to Ligament of Treitz and proximal to distal 1/3 ileum	15 (12.3)	4 (8.9)	11 (14.3)	
Both L4a and L4b	10 (8.2)	3 (6.7)	7 (9.1)	
Not applicable	60 (49.2)	22 (48.9)	38 (49.4)	
Distal disease location at diagnosis*				.635
L1: distal 1/3 ileum ± limited cecal disease	14 (11.5)	4 (8.9)	10 (13)	
L2: colonic disease	13 (10.7)	5 (11.1)	8 (10.4)	
L3: ileocolonic disease	89 (73.0)	35 (77.8)	54 (70.1)	
No distal disease location at diagnosis	6 (4.9)	1 (2.2)	5 (6.5)	
Disease behavior*				.696
B1: nonstricturing/nonpenetrating	109 (89.3)	42 (93.3)	67 (87)	
B2: stricturing	4 (3.3)	1 (2.2)	3 (3.9)	
B3: penetrating	8 (6.6)	2 (4.4)	6 (7.8)	
B2B3: both penetrating and stricturing disease, either at the same time or different times	1 (0.8)	0 (0)	1 (1.3)	
Perianal disease behavior at diagnosis*				.848
Yes	20 (16.4)	7 (15.6)	13 (16.9)	
No	102 (83.6)	38 (84.4)	64 (83.1)	

*P value (χ^2 test) corresponds with the comparison between females and males for each variable. This analysis used baseline cross-sectional data.
†All participants have a final diagnosis of CD. Initial classification of disease may have been ulcerative colitis, IBD unclassified, or CD.

Cytokines and Hormone Bone Age-Z-scores. In females, IL-8 ($P < .001$) and IL-12p70 ($P = .035$) were significantly negatively associated with gonadotropin bone age-Z-scores; IL-8 ($P = .010$), IL-12p70 ($P = .020$), and IFN-γ ($P = .004$) were significantly negatively associated with sex hormone bone age-Z-scores, and IL-8 ($P = .044$) and IFN-γ ($P < .001$) were significantly negatively associated with IGF-1 bone age-Z-scores (Table III, Figure 2). In males, IL-1 beta ($P = .019$) and IL-6 ($P = .025$) were significantly negatively associated with IGF-1 bone age-Z-scores (Table

Table II. Bone age Z-score, height velocity bone age-Z-score, and anthropometric Z-score differences

Characteristics	Male	Female	Sex difference*	Sex difference*	*P* value*
Bone age Z-score	−0.15 ± 1.34 (−5.09 to 4.01)	−1.12 ± 1.25 (−5.43 to 2.97)	0.90	0.41 to 1.39	<.001
Height velocity bone age-Z-score	0.31 ± 1.47 (−3.65 to 5.50)	0.44 ± 1.40 (−2.96 to 4.84)	−0.19	−0.63 to 0.25	.390
Height Z-score difference	−0.27 ± 0.90 (−3.89 to 1.97)	−0.87 ± 0.94 (−3.92 to 1.17)	0.51	0.15 to 0.87	.005
Weight Z-score difference	−0.19 ± 0.67 (−3.3 to 1.62)	−0.62 ± 0.69 (−3.19 to 0.62)	0.37	0.12 to 0.63	.004
BMI Z-score difference	−0.09 ± 0.30 (−1.34 to 0.72)	−0.26 ± 0.27 (−1.12 to 0.32)	0.16	0.05 to 0.27	.006

Values are mean ± SD (range), mean, or 95% CI.
*Sex difference compares the difference in the variable of interest (value in males minus value in females) in a multivariate analysis adjusting for wPCDAI, treatment with biologics, and treatment with immunomodulators. This analysis used longitudinal data collected throughout the entire study period.

Table III. Association between specific cytokines and hormone bone age-Z-scores by sex

Sexes	Cytokine	Hormone	β	95% CI	*P* value*
Males†	IL-1 Beta	IGF-1	−0.235	−0.432 to −0.038	**.019**
Females†	IL-1 Beta	IGF-1	−0.114	−0.262 to 0.034	.131
Sex difference‡	IL-1 Beta	IGF-1	−0.172	−0.369 to 0.026	.088
Males	IL-1 Beta	Sex hormones§	−0.421	−4.170 to 3.329	.826
Females	IL-1 Beta	Sex hormones	0.316	−0.059 to 0.692	.099
Sex difference	IL-1 Beta	Sex hormones	−0.254	−3.540 to 3.031	.880
Males	IL-1 Beta	Gonadotropins¶	−0.026	−1.174 to 1.123	.965
Females	IL-1 Beta	Gonadotropins	0.165	0.051-0.278	.004
Sex difference	IL-1 Beta	Gonadotropins	−0.160	−1.280 to 0.961	.780
Males	IL-12p70	Sex hormones	0.271	−0.673 to 1.216	.574
Females	IL-12p70	Sex hormones	−0.551	−1.017 to −0.086	**.020**
Sex difference	IL-12p70	Sex hormones	0.451	−0.671 to 1.573	.431
Males	IL-12p70	Gonadotropins	0.092	−0.304 to 0.488	.649
Females	IL-12p70	Gonadotropins	−0.162	−0.313 to −0.011	**.035**
Sex difference	IL-12p70	Gonadotropins	0.116	−0.499 to 0.730	.712
Males	IL-13	IGF-1	0.365	0.020-0.711	.038
Females	IL-13	IGF-1	−0.337	−1.034 to 0.360	.343
Sex difference	IL-13	IGF-1	0.819	−0.014 to 1.651	.054
Males	IL-13	Gonadotropins	0.346	−1.016 to 1.709	.619
Females	IL-13	Gonadotropins	−0.748	−1.519 to 0.023	.057
Sex difference	IL-13	Gonadotropins	0.900	−0.876 to 2.676	.321
Males	IL-2	IGF-1	−0.193	−0.623 to 0.238	.381
Females	IL-2	IGF-1	0.145	−0.223 to 0.514	.439
Sex difference	IL-2	IGF-1	−0.437	−0.951 to 0.077	.096
Males	IL-6	IGF-1	−0.212	−0.400 to −0.026	**.025**
Females	IL-6	IGF-1	−0.120	−0.371 to 0.131	.347
Sex difference	IL-6	IGF-1	−0.063	−0.357 to 0.232	.675
Males	IL-6	Sex hormones	−3.293	−9.265 to 2.679	.280
Females	IL-6	Sex hormones	−0.413	−0.847 to 0.021	.062
Sex difference	IL-6	Sex hormones	−2.429	−8.424 to 3.565	.427
Males	IL-8	IGF-1	0.088	−0.114 to 0.289	.393
Females	IL-8	IGF-1	−0.536	−1.057 to −0.015	**.044**
Sex difference	IL-8	IGF-1	0.582	0.025-1.138	**.040**
Males	IL-8	Sex hormones	−2.512	−5.725 to 0.701	.125
Females	IL-8	Sex hormones	−1.918	−3.382 to −0.454	**.010**
Sex difference	IL-8	Sex hormones	0.285	−3.317 to 3.887	.877
Males	IL-8	Gonadotropins	−0.885	−2.200 to 0.431	.187
Females	IL-8	Gonadotropins	−0.615	−0.955 to −0.275	**<.001**
Sex difference	IL-8	Gonadotropins	0.025	−1.352 to 1.401	.972
Males	IFN-gamma	IGF-1	−0.094	−0.210 to 0.023	.115
Females	IFN-gamma	IGF-1	−0.432	−0.598 to −0.265	**<.001**
Sex difference	IFN-gamma	IGF-1	0.346	0.142-0.550	**.001**
Males	IFN-gamma	Sex hormones	0.922	−1.585 to 3.428	.471
Females	IFN-gamma	Sex hormones	−0.837	−1.413 to −0.262	**.004**
Sex difference	IFN-gamma	Sex hormones	1.569	−1.326 to 4.463	.288
Males	TNF-alpha	Sex hormones	−1.522	−4.503 to 1.459	.317
Females	TNF-alpha	Sex hormones	1.010	0.142-1.877	.023
Sex difference	TNF-alpha	Sex hormones	−1.411	−4.504 to 1.681	.371

Bolded *P* values indicate statistically significant negative B values or statistically significant sex differences.
*Every cytokine/hormone combination was examined. This table includes associations with a *P* value of less than .100.
†The β in each of these rows is the regression coefficient corresponding to a specific cytokine indicating its strength of association with the specified hormone bone age-Z-score. The analysis used longitudinal data collected throughout the entire study period. The statistical model also included chronological age, wPCDAI, and treatment with biologics and treatment with immunomodulators to adjust for potential confounding.
‡The β in each of these rows is the regression coefficient corresponding to the specific cytokine-by-sex interaction, which represents the sex difference in the strength of the association between a specific cytokine and a specific hormone bone age-Z-score adjusting for chronological age, wPCDAI, and treatment with biologics and treatment with immunomodulators.
¶Gonadotropins refer to LH and FSH.
§Sex hormones refer to testosterone and estradiol.

III, Figure 2). The association between IL-8 and IGF-1 (P = .040) and between IFN-γ and IGF-1 (P = .001) differed by sex (interaction present) Table III.

Exploratory Analysis Examining Hormones (Bone Age-Z-scores) as Mediators of the Impact of Specific Cytokines on Height Velocity (Bone Age-Z-scores). Findings suggest that IGF-1 mediated the relationship between IL-1*B* and height velocity in males because IL-1*B* was associated with IGF-1 (B = −0.235; 95% CI, −0.432 to −0.038; P = .019) and IGF-1 was also associated with height velocity when adjusting for IL-1*B* (B = 0.635; 95% CI, 0.369-0.902; $P < .001$). IGF-1 mediated the relationship between IL-6 and height velocity in males because IL-6 was associated with IGF-1 (B = −0.213; 95% CI, −0.400 to −0.026; P = .025), and IGF-1 was also associated with height velocity when adjusting for IL-6 (B = 0.663; 95% CI, 0.392-0.933; $P < .001$). We did not identify specific hormones as mediators of the

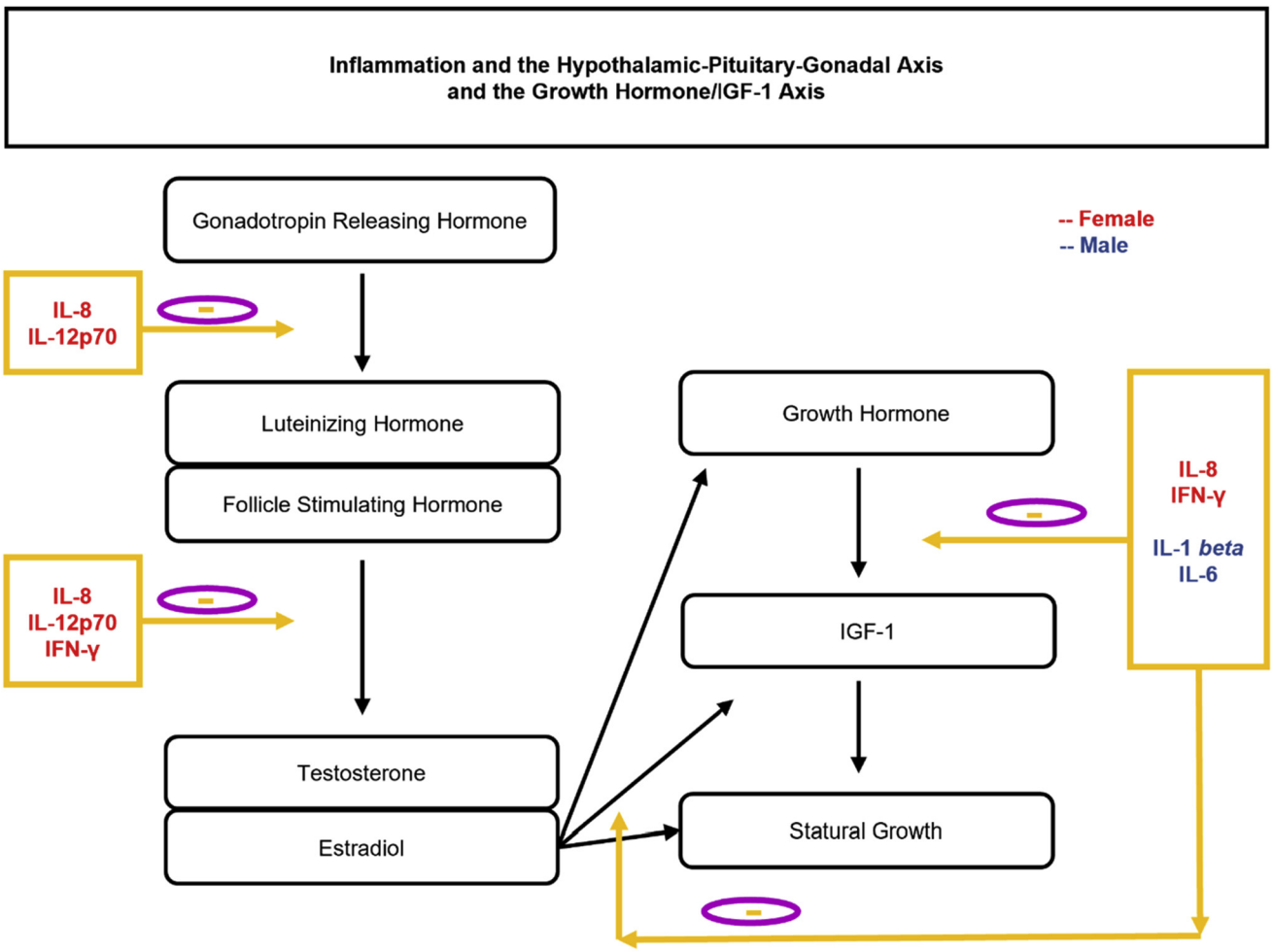

Figure 2. Inflammation and the HPG axis and the growth hormone/IGF-1 axis.

relationship between specific cytokines and height velocity in females.

Discussion

Our longitudinal analyses in this contemporary cohort of pediatric patients with CD receiving current standard treatments showed that, on average, males achieved less standardized height gain with bone age progression than females, reflecting poorer statural growth in males.[1,2] Furthermore, standardized bone age results were lower in females, implying a greater opportunity for females to experience catch up growth.[31] The negative sex-specific associations between specific cytokines and specific hormones suggest that the primary pathway to growth impairment is the growth hormone/IGF-1 axis in males and the HPG axis in females (Figure 2). Taken together, these results seem to support our central hypothesis that inflammation, as measured by specific cytokines, has worse effects on hormones that are important to growth in males vs females, and thus provides a potential mechanism explaining the observed sex difference in growth in CD. Our data suggest that, in males, the inflammatory inhibition of the growth hormone/IGF-1 axis compromises growth, and the lack of bone age delay implies a functional HPG axis resulting in normal rates of epiphyseal closure, limiting the opportunity for catch-up growth. In contrast, females exhibit compromise of the HPG axis, leading to delayed bone age; although the rate of the pubertal growth spurt is attenuated, the growth hormone/IGF-1 axis remains intact, and the delayed bone age progression allows additional time for catch-up growth. Although this theory requires deeper investigation, our results support this theory. Standardized height gain was lower in males with bone age progression and bone age Z-scores were lower in females. For the analyses in which we examined the association between specific cytokine and specific hormone bone age-Z-score for each sex, the model included the specific cytokine and potential confounding factors (ie, biologic treatment, immunomodulator treatment, wPCDAI, and chronological age). As expected, disease duration was longer in males (because males are at greater risk for CD

than females in early childhood) and our study design specified older chronological age criteria for males (because of pubertal timing) to participate in the study.[1,2] Adding disease duration to multivariate models resulted in findings similar to those described and results were similar when adjusting for follow-up time (data not shown). The existing literature supports that sex differences in growth impairment are not driven by the timing of diagnosis of IBD in relation to the timing of the pubertal growth spurt.[4,14]

Standardized height velocity was lower in males, but this finding did not reach statistical significance, possibly secondary to insufficient statistical power; the Growth Study was designed to investigate the underlying mechanisms of sex differences in growth impairment rather than the prevalence of growth impairment. We theorize that small sex differences in standardized height velocity (instantaneous measurement) accumulate over time, leading to greater sex differences in longer term growth as represented by sex differences in height Z-score differences (**Figure 1**).[1,2] Our analyses suggest that IGF-1 mediates the relationship between specific cytokines and height velocity in males, but did not identify specific hormones as mediators of the relationship between specific cytokines and height velocity in females. This analysis assessing hormones as the mediators of the impact of specific cytokines on height velocity is considered exploratory; a limitation of this study is that it is not designed to have adequate power for this analysis, and hormones as mediators in males and females will be addressed in future research. Additional limitations of our work are that we focused on a panel of only 10 specific cytokines, and our study is hypothesis generating rather than hypothesis confirming.

Our findings highlight the importance of assessing an extensive panel of proteins to further decipher the specific molecular pathways leading to growth impairment. The potential of the serum proteome as a source for identification of future diagnostic biomarkers is an emerging theme in the literature.[44,45] Investigators are studying serum proteins to diagnose, classify, predict disease course, map disease activity, decipher drug mechanisms and treatment responses, and understand biological processes in IBD.[44,45] Mapping sex-specific molecular pathways is key to developing new targeted medical treatment strategies that improve statural growth and final adult height, for optimizing treatments in high-risk patients in both sexes, and for laying the foundation for the development of highly needed sex-specific treatment approaches targeting the underlying inflammation for children with CD. Furthermore, it is important to include specific serum inflammatory proteins in risk models to establish which males and which females are at greatest risk for growth impairment refractory to standard of care CD therapies. High-risk patients may benefit from the early introduction of aggressive sex-specific medical therapies to treat the underlying inflammation as only a narrow therapeutic interval is available to intervene to improve growth before long-bone epiphyses fuse. ■

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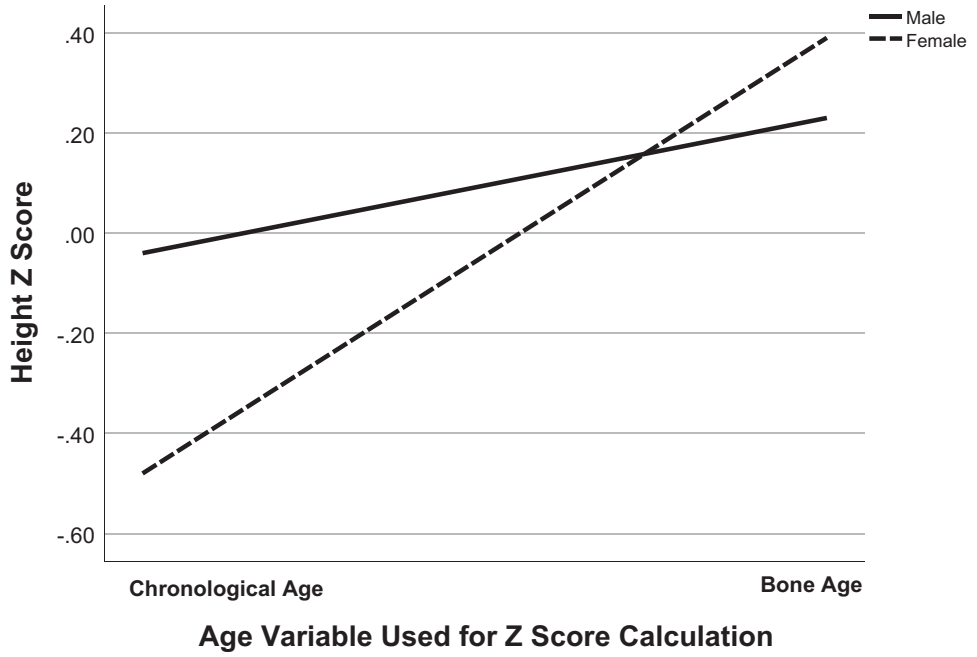

Figure 1. Sex differences in height Z-score differences.