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Fibroblast growth factor-21, body composition, and insulin resistance in pre-pubertal and early pubertal males and females

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Abstract

Objective—Accumulating evidence derived primarily from animal models suggests that fibroblast growth factor-21 (FGF-21) may affect the musculoskeletal system via effects on the capacity of tissues to respond to insulin. A proportion of musculoskeletal properties and underpinnings of promoting/preventing insulin resistance are established early in the pubertal transition. Thus, the objective of this study was to test the hypothesis that insulin resistance and/or obesity will promote greater FGF-21 concentration which will be inversely associated with musculoskeletal parameters [lean mass and bone mineral content (BMC)] in pre-/early pubertal children. Given the sexual dimorphic nature of musculoskeletal development of fat mass accrual, differences by obesity status and sex were also investigated.

Design—Cross-sectional

Patients—Children ages 7–12yr (n=69, 38% male, 48% non-Hispanic black, 45% obese)

Measurements—Fasting FGF-21, glucose and insulin measures were obtained. An estimate of insulin resistance was derived using the homeostatic model assessment of insulin resistance (HOMA-IR). Body composition (BMC, lean mass, and fat mass) was assessed by DXA. Multivariate regression analysis was used to evaluate the influence of FGF-21 on BMC, lean mass, and HOMA-IR as dependent variables. Obesity status was established based on BMI z-score.

Results—FGF-21 concentrations did not differ by obesity status or by sex. There was an inverse association between FGF-21 and BMC among non-obese individuals ($P = 0.01$), and an inverse association between FGF-21 and lean mass among females ($P=0.02$), which were both independent of fat mass. FGF-21 was inversely associated with HOMA-IR in males, but not females ($P=0.04$).

Conclusions—The existence of relationships of FGF-21 with musculoskeletal parameters and insulin resistance raises the possibility of crosstalk between these systems. These findings suggest

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that circulating FGF-21 may differ in its association with bone, lean mass, and insulin resistance depending on sex and weight status.

Keywords

FGF-21; insulin resistance; body composition; pre-/early pubertal children

Introduction

Adverse health outcomes associated with insulin-resistant states (e.g., pre-diabetes, type 2 diabetes) are well-described, and coincide with the pediatric obesity epidemic (1) (2) (3). Numerous studies have established that endocrine factors secreted by adipose tissue (adipokines) influence insulin resistance within the pediatric population (4) (5) (6). However, the extent to which secretory factors emanating from muscle, the body's largest glucose-utilizing tissue, may also exert effects on communicative networks between the musculoskeletal system and insulin dynamics is poorly understood.

Fibroblast growth factor-21 (FGF-21) is a hormone that may partly mediate the ability of skeletal muscle to maintain glucose homeostasis. Although primarily secreted by the liver and adipose tissue, FGF-21 has recently been identified as myokine exerting(7) important systemic actions including facilitating metabolic adaptation in the fasted state (8) (9) (10) and regulating glucose uptake (11), (12) (13). Higher circulating FGF-21 levels in humans has been observed among insulin-resistant and obese individuals relative to their insulin-sensitive and normal weight peers (11) (14) (15). In addition to having a role in glucose homeostasis, fuel utilization pathways converge with anabolic growth processes suggesting FGF-21 may be important for developmental aspects of the musculoskeletal system (e.g. myogenesis, thermogenesis, osteogenesis).(16) (17).

Metabolic insulin responses undergo substantial changes during puberty, particularly among girls. Most notably the increase in estradiol from the ovaries increases lipid mobilization, drives fat deposition, and coincides with a heightened state of insulin resistance, manifesting by the second Tanner stage. In males, the hormonal changes are more sequential and the insulin-resistant state is shorter and less pronounced. These changes may play an important role in coordinating musculoskeletal adaptations associated with the rapid period of growth in puberty. Therefore, evaluating the relationships between FGF-21, body composition and insulin resistance during pre-/early puberty is warranted. Our overarching hypothesis is that elevated FGF-21 concentrations among insulin resistant and obese children can adversely affect musculoskeletal development. Early pubertal children are an ideal model to test this hypothesis, as this represents a stage characterized by substantial metabolic changes in preparation for rapid linear growth. Further, as influential factors in rate and/or pattern of growth, weight status and sex may impact FGF-21 actions. The objectives of this study were to 1) characterize the relationship between FGF-21 levels in circulation with quantitative aspects of the musculoskeletal system (i.e., bone mineral content and lean mass) and insulin resistance in pre-pubertal and early pubertal children, and 2) investigate differences by obesity status and sex.

Materials and Methods

Participants included a convenience sample of pre-pubertal and early pubertal children aged 7–12 years who participated in a variety of research protocols within the Department of Nutrition Sciences at the University of Alabama at Birmingham (UAB) from 2011 to 2013 and had stored blood samples for measurement of FGF-21. Samples were derived from a dietary weight loss intervention [described in Casazza et al (18)], and from two observational studies investigating body composition and metabolic health in boys (NCT02040740) and girls (NCT01410643). All data used in the analysis were derived from the baseline assessment if enrolled in a study with multiple collection points and blood draw protocols were the same across all studies. Participants were recruited using newspaper advertisements, posted flyers, various community partnerships, by word-of-mouth and through local radio advertisements. Exclusion criteria were medical diagnoses and/or current use of medications known to affect body composition, lipid or glucose metabolism, or blood pressure (e.g., diabetes; impaired fasting glucose; use of thyroid medication, diuretics, beta-blockers, thiazolidinediones, etc.); an allergy to EMLA cream, which was used for topical anesthesia prior to venipuncture in study participants; and history of an eating disorder(s). The nature of the studies was carefully explained to each participant and guardian(s), and informed assent and consent, respectively, were obtained. All research protocols were approved by the Institutional Review Board for human subjects at UAB. All measurements were performed at the Clinical Research Unit (CRU) and the Department of Nutrition Sciences at UAB.

Blood/serum analyses

Blood was drawn after an overnight fast to obtain glucose, insulin, and FGF-21. Serum concentrations of FGF-21 were measured using a commercially-available enzyme linked immunoassay (Millipore Corporation, Billerica, MA). All samples were processed immediately upon completion of blood draw to extract serum samples and subsequently stored at –80 degrees until measurement of the analytes of interest in batched assays in accordance with assay specifications. The inter- and intra-assay coefficients of variation (CV) were < 10%. Glucose was measured in 3 µl sera with the glucose oxidase method using a SIRRUS analyzer (inter-assay CV 2.56%). Insulin was analyzed using a TOSOH AIA-600 II Automated Immunoassay Analyzer (TOSOH Bioscience, South San Francisco, CA, USA). Minimum assay sensitivity was 0.5uU/ml, mean intra-assay CV was 4.69%, and inter-assay CV was 6.0%. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), based on fasting insulin and glucose concentrations⁹.

Height, Weight, BMI Z-Score

Height (Heightronic 235; Measurement Concepts, Snoqualmie, WA, USA) and weight (Scale-tronix 6702W; Scale-tronix, Carol Stream, IL, USA) were measured in minimal clothing without shoes. BMI z-score was calculated using age- and sex-specific CDC growth charts¹¹.

Body Composition

Whole-body DXA scanning was used to assess fat mass, lean soft tissue mass, bone mineral content (BMC), and bone mineral density (BMD) using an iDXA instrument (GE-Lunar, Madison, WI) with pediatric software.

Pubertal status

Pubertal stage was determined by direct observation according to the staging based on the criteria of Marshall and Tanner (19) (20). One composite number was assigned for Tanner staging, representing the higher of the two values defined by breast/testes development and/or pubic hair.

Statistics

Descriptive statistics (mean $\pm$ standard error) were determined for the overall sample and for stratified groups according to obesity status (z-score < -1.64) and sex, respectively. Two-sample *t*-tests were used to evaluate group differences for normally distributed variables and Wilcoxon rank sum tests for non-normally distributed variables. FGF-21 was not normally distributed, thus median and interquartile range were calculated for this variable and used for descriptive statistical analysis. Multiple linear regression analysis was performed with log-transformed FGF-21 as the independent variable and body composition parameters (lean mass and BMC) and HOMA-IR as dependent variables in individual models. For each dependent variable, multiple models were evaluated. The first model was adjusted for race, pubertal stage, and BMI z-score. In addition, given that adipose tissue secretes FGF-21 and is strongly linked to muscle and bone mass, we further adjusted for total fat in additional models. Based on variation due to sex and weight status on growth and developmental patterns and glucose utilization, particularly during early puberty, we hypothesized that the contribution of FGF-21 to these parameters may be altered in the obese state and/or exhibit sexual dimorphism. Thus, models were also analyzed stratified by obesity status and sex. All statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary NC) and a two-sided *P*-value of 0.05 was considered statistically significant.

Results

Participant characteristics are presented in Table 1 for the total sample (n=69) as well as stratified by weight status and sex. The mean age of the children (38% male) was 9.5 ± 1.8 years. Approximately half (49%) were non-Hispanic white, 48% were non-Hispanic black, and 3% were Hispanic white. Further, based on BMI z-score, approximately 45% of the children were obese.

As compared to non-obese participants, obese participants were more likely to be non-Hispanic black (63% versus 37%; $P < 0.05$) and have greater lean mass ($P = 0.0001$). Although obese participants had greater BMD ($P = 0.0001$) relative to non-obese individuals, a difference in BMC failed to reach statistical significance ($P = 0.09$). Glucose-related parameters differed by weight status such that obese children had higher insulin concentrations ($P = 0.01$), lower glucose concentrations ($P = 0.05$), and therefore greater HOMA-IR ($P = 0.01$) than non-obese individuals. Compared to male participants, female

participants were less reproductively mature ($P < 0.001$) and had greater total body fat ($P = 0.01$). Females also had higher insulin and HOMA-IR (each $P = 0.01$). FGF-21 concentrations did not differ by sex or obesity status defined by BMI z-score, nor was it associated with total fat mass (data not shown). There was also no difference in FGF-21 concentrations when stratifying by adiposity level defined according to percent fat mass (males $\leq 25\%$, females $\leq 30\%$ (21)).

We next evaluated the independent relationship between FGF-21 and lean mass, bone mass and insulin resistance, respectively. The relationship between FGF-21 and lean mass is shown in Table 2. There were no associations of FGF-21 with lean mass in the overall study sample. Adjusting for age, pubertal stage and BMI z-score, a significant inverse association between FGF-21 and lean mass among females ($P = 0.02$) was observed, which persisted after further adjustment for fat mass. In addition, stratified analysis by obesity status showed an inverse association between FGF-21 and lean mass in the obese group after adjustment for fat mass ($P = 0.03$).

Associations of FGF-21 with BMC are shown in Table 3. There were no associations of FGF-21 with BMC in the overall sample or when stratified by sex. In models stratified by weight status, there was an inverse association between FGF-21 and BMC among non-obese individuals ($P = 0.01$). This association remained significant after further adjusting for fat mass ($P = 0.01$).

The relationship between FGF-21 and HOMA-IR is depicted in Table 4. FGF-21 was not significantly associated with HOMA-IR in the full study sample or when the sample was stratified by obesity status. When stratified by sex, FGF-21 was inversely associated with HOMA-IR in males independent of race, pubertal stage, and BMI z-score ($P = 0.02$), and persisted after adjustment for fat mass ($P = 0.04$).

Discussion

While there is accumulating evidence that FGF-21 is a potent regulator of various aspects of metabolism, including insulin dynamics and oxidative potential and fat thermogenesis (e.g., browning of fat) in animal models, the relationship between circulating levels of FGF-21 and growth parameters in humans is less clear, particularly in children. FGF-21 action may be particularly unique in its contribution to metabolism in pre-pubertal and early pubertal children. In preparation for reproductive development and linear growth, insulin sensitization capacity and/or resistance are integral in the partitioning of muscle, bone and adipose tissue. Despite this, little is known about the associations of FGF-21 with body composition and insulin resistance in pre-pubertal and early pubertal children, which has driven this investigation. In this study of pre-pubertal and early pubertal children, FGF-21 concentrations were inversely associated with lean mass among females and obese individuals, suggesting a potential attenuation of the association of FGF-21 with lean mass in the setting of increasing adiposity. This is further supported at least in part by the inverse association of FGF-21 with BMC in non-obese individuals only. Further, FGF-21 concentrations were inversely associated with insulin resistance in males, who had significantly less fat mass than females.

Unique findings of this study were the sexual dimorphisms displayed in the relationship between FGF-21 and lean mass and FGF-21 and insulin resistance. FGF-21 was inversely associated with lean mass in females but not males. Additionally, when stratified by sex, we found an inverse association of FGF-21 with HOMA-IR in boys but not girls. However, in accordance with findings by Reinehr et al in children, we found no association of HOMA-IR with FGF-21 levels in the overall sample. While further investigations into interaction between circulating FGF-21 and growth/sex hormones are necessary to support an active role, FGF-21 has been shown to stimulate glucose uptake in muscle cells and is thought to be a novel marker of mitochondrial health in skeletal muscle tissue (25) (24). As muscle is the largest glucose-utilizing tissue in the body, differences in the association of FGF-21 with lean mass by sex may represent variation in insulin dynamics at the level of skeletal muscle tissue in girls as compared to boys.. The hormonal response (e.g. insulin and estradiol) in females is necessary to maintain reproductive fecundity and secure appropriate fat stores for reproduction, whereas as in males synthesis and release of hormones (e.g. insulin and testosterone) favors lean mass accretion. Not only do girls gain fat more readily than boys, insulin resistance occurs earlier, the magnitude is greater and the resolution less complete (28) (29) (30) (31) (32). Thus the physiologic promotion of fat deposition by estrogen, and in context of greater insulin resistance, which is evident as early as just prior to the onset of puberty (30), cumulatively could account for a substantial sex difference in the relationships of FGF-21 with lean mass and insulin resistance.. Future studies will need to examine the change in FGF-21 concentrations in relation to hormonal fluctuations in females particularly in terms of fat distribution and estrogen receptor subtype, as well as utilize more specific measures of insulin dynamics and include a larger sample size across the pubertal transition to dissect these possibilities.

It is important to acknowledge that FGF-21 is produced and secreted by not only skeletal muscle, but also by the liver and adipose tissue, and action in circulation may vary according to tissue of origin (34). Depending upon the physiological context, local tissue concentration may be divergent from the serum level, and substantial differences between auto- and endocrine effects needs to be considered (35). We also acknowledge the functional capacity of FGF-21 as a myokine is influenced at least in part by the interaction with adipokines; however, controlling for fat mass, as well as stratifying analysis by weight status, may have likely limited confounding. The adipokine, leptin, which is increased in the obese state, has been shown to positively correlate with FGF-21; and another adipokine, adiponectin, which is decreased in the obese state, acts directly on bone to induce human osteoblast proliferation and differentiation, and to indirectly increase osteoclast formation. Alteration in native adipokines with high adiposity levels may interfere with communicative signals between muscle (and even liver and adipose tissue) and bone. In animal studies, an inhibitory effect of FGF-21 bone formation has been shown (17). However, these studies may not directly translate to humans in physiologic states. One study of healthy adults showed a positive association between plasma FGF-21 levels and both total and spine BMD in women but not men (33). In the current study, an association between FGF-21 and BMC was not apparent in males or females. However, FGF-21 was inversely associated with BMC in non-obese children only after controlling for fat mass. As muscle and bone adapt in parallel, lack of detection of a relationship between FGF-21 and BMC in obese participants may suggest that

obesity disrupts effects of FGF-21 on bone metabolism. Further studies should examine the associations of FGF-21 and bone qualitative measures in obese as compared to non-obese children, particularly during accelerated growth.

Contrary to findings of some (14, 15) but not all studies (36), serum FGF-21 concentrations did not differ by obesity status. The discrepancy amongst studies could be due to the different cut points used to define obesity (Reinehr et al used 30 kg/m² to define obesity; the adult study combined overweight and obese subjects). We also investigated whether FGF-21 levels differed by obesity status as defined by adiposity level (> 30 and 25% for girls and boys, respectively), and found no difference. Another potential explanation is the varied range of weight status within groups and the inclusion of multiple race/ethnicities in our study as compared to prior studies. Whereas for this analysis, normal and overweight subjects were combined in a multi-racial cohort, the study by Giannini et al comprised a 100% Caucasian sample, excluding subjects between the 87th and 95th percentile. Furthermore, the current study included pre-pubertal and early pubertal children and may not be generalizable across the life span. Although growth-velocity is the lowest in the pre-pubertal group, which represented 81% of the cohort, compared to early-to-mid pubertal children, manifestation of metabolic changes may not yet be apparent. Therefore, lack of significance may be also attributed to only minor changes in body composition as expected in children without pubertal development.

While this study has notable strengths, such as representation of an under-studied group (pre-pubertal and early pubertal children), as well as a relatively robust measure of body composition using DXA, we acknowledge our limitations. The cross-sectional nature of the study precludes causal inference. The relatively small sample size, representing a mixed population (variance in race, sex and body habitus) may have precluded the identification of statistically significant associations of modest effect size, particularly upon stratification of subgroups. Further, our sample, while in line with previous studies evaluating body composition parameters in this geographic region, has a greater obesity prevalence than the national average. However, it represents to our knowledge the first assessment of the relationship between musculoskeletal parameters, FGF-21 and insulin resistance in this age group in a population which may have increased risk of adverse musculoskeletal health.

While the mechanisms and the extent to which crosstalk exists involving FGF-21, musculoskeletal parameters, and insulin resistance and differences by obesity status and sex in humans are not clear, these findings raise some intriguing possibilities. While preliminary, these findings suggest that although absolute levels by sex and obesity status did not differ, the associations of FGF-21 with lean mass, bone content and insulin resistance may differ by sex and obesity. The existence of relationships of FGF-21 with musculoskeletal parameters and insulin resistance raises the possibility of crosstalk between these systems through paracrine mechanisms. These novel findings are important to further investigate in peri-pubertal children given the recent appreciation of FGF-21 as a myokine and the unique metabolic milieu characteristics of the pubertal maturation.

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Descriptive characteristics (means and standard deviations or median and interquartile range) of the overall sample, by sex and by obesity status (non-obese < 1.64 BMI z-score; obese 1.64 BMI z-score (38))

Table 1

Variable	Overall (n=69)	Non-Obese (n=38)	Obese (n=31)	Males (n=26)	Females (n=43)
Age (yrs)	9.5 ± 1.8	9.2 ± 2.0	9.8 ± 1.3	9.3 ± 2.2	9.7 ± 1.4
Tanner stage (n) [‡]					
1	56	28	28	14	42
2	13	10	3	12	1
Race (% non-Hispanic Blacks)	48	37	63***	58	42
Height (cm)	139.5 ± 12.4	135.3 ± 11.6	144.6 ± 11.4**	139.5 ± 10.5	139.5 ± 13.5
Weight (kg)	44.1 ± 17.0	32.7 ± 8.4	58.0 ± 14.3*	39.5 ± 12.6	46.9 ± 18.8
BMI Z-Score	1.1 ± 1.2	0.3 ± 1.0	2.2 ± 0.3*	0.9 ± 1.0	1.2 ± 1.3
Percent fat	32.6 ± 9.7	25.5 ± 6.1	41.4 ± 4.9*	28.7 ± 8.4	35.0 ± 9.8**
Fat mass (kg)	15.5 ± 9.4	8.6 ± 3.9	24.0 ± 6.9*	12.0 ± 6.8	17.6 ± 10.2**
Lean mass (kg)	26.7 ± 7.7	22.6 ± 4.9	31.7 ± 7.5*	25.7 ± 6.1	27.3 ± 8.5
BMC (kg)	1.7 ± 2.7	1.2 ± 0.3	2.3 ± 4.0	1.4 ± 0.3	1.9 ± 3.5
BMD (g/cm ³)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1*	0.9 ± 0.1	0.9 ± 0.1
FGF-21 (pg/ml)	156.0 [59.0, 254.0]	167.0 [54.0, 260.0]	143.0 [59.0, 237.0]	137.0 [39.0, 260.0]	157.0 [67.0, 237.0]
Insulin (nmol/L)	45 ± 31.2	28.8 ± 16.8	66.0 ± 33.0*	31.8 ± 18.6	53.4 ± 34.8**
Glucose (mmol/L)	4.9 ± 0.4	5.0 ± 0.4	4.8 ± 0.4***	4.9 ± 0.4	4.9 ± 0.5
HOMA-IR	1.6 ± 1.2	1.1 ± 0.7	2.4 ± 1.3*	1.1 ± 0.7	1.9 ± 1.3**

BMC=Bone mineral content; BMD=Bone mineral density; FGF-21=Fibroblast growth factor-21; HOMA-IR=Homeostatic model assessment model of insulin resistance;

[‡] based on criteria of Marshall and Tanner (19, 20);

* $P < 0.0001$,

** $P < 0.01$,

*** $P < 0.05$

Table 2

Multiple linear regression analysis: lean mass as dependent variable and log-transformed FGF-21 the independent variable; overall, by sex, and by obesity status (non-obese < 1.64 BMI z-score; obese 1.64 BMI z-score)

Lean Mass										
Model	Overall (n=69)		Non-Obese (n=38)		Obese (n=31)		Males (n=26)		Females (n=43)	
	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value
Covariates *	-954.7	0.109	-967.5	0.067	-1386.7	0.242	-188.5	0.810	-2198.8	0.015
Covariates * +Fat Mass	-675.8	0.062	-641.6	0.132	-1331.4	0.032	215.7	0.737	-1268.1	0.015

PE=parameter estimate;

* race, pubertal stage, and BMI z-score for overall and sex-stratified models; race and pubertal stage for obesity status-stratified models

Multiple linear regression analysis: log-transformed bone mineral content as dependent variable and log-transformed FGF-21 the independent variable; overall, by sex and by obesity status (non-obese < 1.64 BMI z-score; obese 1.64 BMI z-score)

Table 3

Bone Mineral Content										
Model	Overall (n=69)		Non-Obese (n=38)		Obese (n=31)		Males (n=26)		Females (n=43)	
	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value
Covariates *	-0.07	0.187	-0.11	0.007	-0.05	0.715	-0.01	0.822	-0.16	0.090
Covariates * +Fat Mass	-0.06	0.239	-0.09	0.011	-0.05	0.723	0.01	0.797	-0.13	0.167

PE=parameter estimate;
* race, pubertal stage, and BMI z-score for overall and sex-stratified models; race and pubertal stage for obesity status-stratified models

Table 4

Multiple linear regression analysis: homeostasis model assessment of insulin resistance as dependent variable and log-transformed FGF-21 the independent variable; overall, by sex and by obesity status (non-obese < 1.64 BMI z-score; obese ≥ 1.64th BMI z-score)

Homeostatic Model Assessment of Insulin Resistance										
Model	Overall (n=69)		Non-Obese (n=38)		Obese (n=31)		Males (n=26)		Females (n=43)	
	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value	PE	P-Value
Covariates *	-0.12	0.228	-0.12	0.161	-0.15	0.460	-0.21	0.022	-0.21	0.189
Covariates * +Fat Mass	-0.09	0.301	-0.11	0.213	-0.16	0.379	-0.18	0.036	-0.11	0.436

PE=parameter estimate;

* race, pubertal stage, and BMI z-score for overall and sex-stratified models; race and pubertal stage for obesity status-stratified models