The Ratio of Parathyroid Hormone to Vitamin D Is a Determinant of Cardiovascular Risk and Insulin Sensitivity in Adolescent Girls

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Abstract

Background: Vitamin D insufficiency and higher testosterone are common in obese girls and may adversely affect glucose homeostasis and cardiovascular risk. Data are conflicting regarding the impact of parathyroid hormone (PTH) on these factors. Our objective was to determine associations of 25-hydroxyvitamin D (25-OHD), PTH, and testosterone with measures of glucose homeostasis and cardiovascular risk in adolescent girls after controlling for regional adiposity, with the hypothesis that lower 25-OHD, a higher PTH or PTH/25-OHD ratio, and higher testosterone would be associated with lower insulin sensitivity and greater cardiovascular risk.

Methods: A total of 15 obese girls and 15 matched normal weight controls (12–18 years) underwent fasting measurements of 25-OHD, PTH, testosterone, sex hormone-binding globulin (SHBG), high-sensitivity C-reactive protein (hsCRP), oral glucose tolerance testing, and quantification of visceral (VAT) and subcutaneous (SAT) fat by magnetic resonance imaging (MRI).

Results: There were no associations of 25-OHD with measures of glucose homeostasis or hsCRP. In contrast, PTH and PTH/25-OHD were associated negatively with homeostasis model assessment of insulin resistance (HOMA-IR) and positively with quantitative insulin sensitivity check index (QUICKI) in obese girls but not controls. These associations remained significant after controlling for body mass index standard deviation score (BMI-SDS), but not for VAT. On regression modeling, PTH/25-OHD was positively associated with hsCRP after controlling for BMI-SDS or VAT. Free testosterone positively predicted the corrected insulin response.

Conclusions: In obese girls, PTH/25-OHD is positively associated with measures of insulin sensitivity and hsCRP. Further studies are needed to investigate the relationship between PTH and glucose homeostasis in obesity.

Introduction

OBESITY IS PREVALENT IN CHILDREN and is a high-risk condition for impaired glucose homeostasis and type 2 diabetes. Determinants of impaired glucose homeostasis in obese children include body mass index (BMI) and measures of regional adiposity, such as visceral adipose tissue (VAT).¹ In addition, a high prevalence of vitamin D deficiency in obese children has raised the intriguing possibility of a relationship between vitamin D deficiency and glucose homeostasis. Another possible determinant of impaired glucose homeostasis in obese adolescent girls is higher testosterone (which may track with lower vitamin D levels^{2,3}), which has not been studied in an adolescent population in relation to measures of insulin sensitivity.

This is relevant given that hyperinsulinemic–euglycemic clamp studies demonstrate increased insulin resistance following testosterone administration in female to male transsexuals.⁴

More than half of obese children and adolescents in the United States may have vitamin D insufficiency.^{5–7} Vitamin D is stored in fat depots,^{8–10} and it is uncertain whether relatively low circulating vitamin D in obesity has functional consequences or simply reflects increased storage in fat. Whereas some studies have reported inverse associations between 25-hydroxyvitamin D (25-OHD) and insulin resistance parameters, independent of aggregated measures of adiposity such as BMI and body fat,^{11,12} others have shown no independent association between 25-OHD and glucose homeostasis after controlling for fat mass.^{13,14}

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In addition, parathyroid hormone (PTH) is inversely associated with vitamin D, and data are conflicting regarding the impact of PTH on glucose homeostasis. Some studies suggest a positive association between PTH and measures of insulin resistance,^{15,16} and with high sensitivity C-reactive protein (hsCRP),¹⁷ a marker of chronic inflammation and cardiovascular risk. In contrast, PTH increases production of 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$], which may play an important positive role in β -cell function and insulin sensitivity.^{18–22}

In the current investigation, we aimed to determine whether 25-OHD, PTH or the ratio of PTH/25-OHD,^{17,23} and testosterone predict insulin resistance and cardiovascular risk markers in a well-characterized group of obese and normal weight adolescent girls after controlling for regional fat mass.

Subjects and Methods

Subject selection

We studied 30 adolescent girls (15 obese and 15 normal weight) between 12 and 18 years, matched for race, ethnicity, and bone age (within a year). Clinical characteristics (but not vitamin D, PTH, or testosterone levels) have been reported previously.^{1,24} Subjects were matched for bone age rather than chronological age because obese girls typically mature earlier than normal weight girls. Bone age was assessed using methods of Greulich and Pyle.²⁵ Obese girls had a BMI >95th percentile for age, whereas normal weight girls had a BMI between the 15th and 85th percentiles. Three obese girls and 2 normal weight girls had not attained menarche; however, all subjects were in puberty. None of the subjects had clinical features suggestive of hyperandrogenism. Exclusion criteria included pregnancy, use of medications that may affect body composition and insulin resistance (such as estrogen, progesterone and glucocorticoids), significant weight gain or loss (more than 2 kg) within 3 months of the study, diabetes mellitus, and thyroid disorders. None of the subjects were being treated for vitamin D deficiency, and similar proportions of subjects reported taking calcium and vitamin D supplements intermittently (46.7% of controls vs. 53.3% of obese girls). Subjects were recruited through mass mailings to pediatricians and advertisements in community newspapers and within the Partners HealthCare network. The Institutional Review Board of Partners HealthCare system approved the study. Informed assent and consent were obtained from subjects and parents, respectively, when subjects were <18 years old, and informed consent was obtained from girls 18 years old.

Experimental protocol

Weight was measured to the nearest 0.1 kg on a single electronic scale at the Clinical Research Center (CRC) of Massachusetts General Hospital. Height was measured using a single stadiometer to the nearest 0.1 cm using an average of three measurements. Subcutaneous adipose tissue (SAT) and VAT were assessed at the lumbar 4–5 level using magnetic resonance imaging (MRI). All subjects had a fasting oral glucose tolerance test (OGTT) using a 1.75 g/kg (maximum 75 g) glucose load. Blood was drawn at 0, 30, 60, 90, and 120 min for glucose and insulin levels. The 0' blood sample

was also assessed for testosterone, sex hormone-binding globulin (SHBG), 25-OHD, PTH, hsCRP (a marker of cardiovascular risk), calcium, and creatinine. The groups did not differ for season of testing. Blood sampling occurred during spring in 3 obese subjects and controls each, during the summer months in 10 obese subjects and 8 controls, during the fall in 1 obese subject and 2 controls, and during winter in 1 obese subject and 2 controls. We have previously reported hsCRP levels for these subjects.²⁴ Dual-energy X-ray absorptiometry (DXA; Hologic 4500, Waltham, MA) was used to assess fat mass.

Biochemical assays

Levels of testosterone, SHBG, 25-OHD, and PTH were assayed by Labcorp using electrochemiluminescence immunoassays (total testosterone, Roche Diagnostics, Indianapolis, IN, limit of detection 2.5 ng/dL; SHBG, Roche Diagnostics, Indianapolis, IN, limit of detection 0.35 nmol/L; 25-OHD, DiaSorin Inc., Stillwater, MN, sensitivity <4.0 ng/dL; PTH, Roche Diagnostics, Indianapolis, IN, limit of detection 1.2 pg/mL). Free androgen index was calculated using the following formula: [Total testosterone (ng/dL)*3.47/SHBG (nmol/L)].²⁶ We also calculated free testosterone using the law of mass action (www.issam.ch/freetesto.htm/).²⁶ We used radioimmunoassay (RIA) to assess insulin [Diagnostic Products Corp, Los Angeles, CA; intraassay coefficient of variation (CV) 3.1%–9.3%, sensitivity 1.2 $\mu\text{IU/mL}$], whereas glucose, calcium, and creatinine levels were assayed in the hospital laboratory using published methods. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: [Fasting glucose in mmol/L*fasting insulin in uU/mL]/22.5. Corrected insulin response (CIR) was calculated as: (Insulin at glucose peak $\times$ 100)/[(glucose peak) (glucose peak – 70)]. Quantitative insulin sensitivity check index (QUICKI) was calculated as: $1/[\log \text{fasting glucose} + \log \text{fasting insulin}]$.^{27,28} Methods used to assay hsCRP have been previously described.²⁴ All samples were stored at -80°C until analysis and analyzed in duplicate.

Statistical methods

Data are described as mean $\pm$ standard deviation (SD). Version 9.0.0 of the JMP program (SAS Institute, Cary, NC) was used for statistical analysis. We used the Student *t*-test for analysis of differences between means for two groups. When data were not normally distributed, these were log transformed before performing the *t*-test or a nonparametric test (Wilcoxon rank sum test) was used. A *P* value of <0.05 was used to denote significance.

Pearson correlations were conducted to determine associations between variables for normally distributed data or after logarithmic conversion of data to approximate a normal distribution. Spearman correlations were conducted when variables were not normally distributed, even after logarithmic conversion (necessary within normal weight controls for insulin measures and hsCRP). We also performed stepwise regression modeling to determine independent predictors of measures of insulin resistance and sensitivity and of hsCRP with (1) PTH/25-OHD, (2) free testosterone or SHBG, and (3) BMI-SDS or VAT entered into the model (*P* value of 0.10 used to enter or leave the model).

Results

Clinical characteristics

Table 1 describes the clinical characteristics of the obese and normal weight groups. The obese girls did not differ from controls for bone age or Tanner stage, but per study design had higher BMI, total and percent body fat, VAT, and SAT values than controls. Obese adolescent girls also had higher measures of hsCRP, insulin area under the curve (AUC), fasting insulin/glucose, HOMA-IR, and CIR and lower QUICKI values than controls. 25-OHD, PTH, the ratio of PTH/25-OHD, total testosterone, free androgen index, and free testosterone did not differ between groups, although SHBG levels were lower in obese girls.

Associations of regional fat measures, free testosterone, PTH, and vitamin D with insulin measures and with hsCRP

Table 2 shows associations of BMI- standard deviation score (SDS), regional body fat (VAT and SAT), free testosterone, SHBG, PTH, 25-OHD, and PTH/25-OHD with measures of insulin resistance and sensitivity and hsCRP. BMI-SDS, VAT, and SAT were associated positively with insulin AUC, fasting insulin/glucose ratio, and HOMA-IR, and inversely with QUICKI for the group taken as a whole,

but these associations did not persist within the individual groups. BMI-SDS, VAT, SAT, and SHBG were associated positively with hsCRP for the group as a whole, and within obese girls.

There were no associations of 25-OHD with any insulin measures. Instead, we observed inverse associations of PTH and PTH/25-OHD with fasting insulin/glucose ratio and HOMA-IR, and positive associations with QUICKI within obese girls alone. Free testosterone (or the free androgen index), in contrast, was associated positively with fasting insulin/glucose ratio and HOMA-IR and inversely with QUICKI within normal weight controls and positively associated with CIR within obese girls. We also observed positive associations of free testosterone with CIR for the group as a whole. Associations were overall similar for the free androgen index or free testosterone with covariates, and only associations with free testosterone are reported.

Regression modeling to determine independent predictors of insulin resistance and sensitivity and hsCRP

Because associations of free testosterone and PTH (or PTH/25-OHD) with measures of insulin resistance and sensitivity and with hsCRP may be confounded by covariates such as BMI-SDS or regional fat measures, we next

TABLE 1. CLINICAL CHARACTERISTICS OF OBESE AND NORMAL WEIGHT ADOLESCENT GIRLS

	Controls (n=15)	Obese subjects (n=15)	P
Age (years)	15.9±1.6	14.0±1.9	0.006
Bone age (years)	15.8±1.8	15.1±1.9	NS
Tanner stage (breasts)	4.5±1.1	4.3±1.0	NS
BMI (kg/m ²)	21.7±1.9	34.4±7.1	<0.0001
Body composition measurements			
Total fat mass (kg) (DXA)	16.5±3.4	39.2±13.6	<0.0001
Percent body fat	28.0±4.0	40.5±6.0	<0.0001
Subcutaneous adipose tissue (SAT) (g)	137.9±49.3	409.4±159.4	<0.0001
Visceral adipose tissue (VAT) (g)	19.0±7.5	42.7±17.0	<0.0001
Oral glucose tolerance test (OGTT)			
Fasting glucose (mg/dL)	83.9±3.8	87.1±9.2	NS
Fasting insulin (μIU/mL) ^a	4.6 [3.8–5.3]	11.3 [6.1–13.1]	0.008
Insulin AUC-OGTT (μIU/mL·120 ⁻¹) ^a	4620 [3642–6237]	8682 [6147–14,756]	0.001
Fasting insulin/glucose (μU/mL·Mg/dL ⁻¹) ^a	0.056 [0.043–0.064]	0.138 [0.066–0.161]	0.01
HOMA-IR ^a	0.933 [0.812–1.135]	2.235 [1.171–2.812]	0.004
Corrected insulin response (CIR) ^a	0.744 [0.479–1.111]	1.046 [0.838–1.328]	0.04
QUICKI ^a	0.388 [0.376–0.397]	0.338 [0.327–0.374]	0.004
Inflammatory cardiovascular risk markers			
hsCRP (mg/L) ^a	0.3 [0.2–0.8]	3.6 [0.4–10.1]	0.006
Biochemical parameters			
Testosterone (ng/dL)	31.7±11.0	24.2±11.7	NS
Sex hormone-binding globulin (nmol/L)	53.1±23.7	27.5±9.6	0.001
Free androgen index	2.4±1.1	3.3±1.9	NS
Free testosterone (ng/dL)	0.451±0.185	0.484±0.245	NS
25(OH) vitamin D (ng/mL)	22.0±8.4	22.6±11.6	NS
PTH (pg/mL)	37.6±16.5	46.5±16.1	NS
PTH/25(OH) vitamin D (*10 ⁻³)	0.37±0.09	0.22±0.13	NS
Calcium (mg/dL)	9.67±0.32	9.57±0.22	NS
Creatinine (mg/dL)	0.86±0.22	0.78±0.14	NS

^aWilcoxon rank sum test used to compare groups; data presented as median and interquartile range.

NS, not significant; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; AUC, area under the curve; HOMA-IR, homeostasis model of insulin resistance; QUICKI, quantitative insulin sensitivity check index; hsCRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone.

TABLE 2. CORRELATIONS OF BMI-SDS, REGIONAL FAT STORES, FREE TESTOSTERONE, 25(OH) VITAMIN D, AND PTH WITH INSULIN PARAMETERS, AND hsCRP

	<i>Insulin AUC*</i>		<i>Insulin/glucose*</i>		<i>HOMA-IR*</i>		<i>QUICKI*</i>		<i>CIR*</i>		<i>hsCRP*</i>	
	r	P	r	P	r	P	r	P	r	P	r	P
BMI-SDS												
All	0.61	0.004	0.52	0.004	0.55	0.002	-0.55	0.002	0.42	0.02	0.78	<0.0001
Obese	0.34	NS	0.21	NS	0.21	NS	-0.21	NS	0.48	0.08	0.86	<0.0001
Controls	-0.39	NS	0.39	NS	0.31	NS	-0.31	NS	-0.05	NS	0.39	NS
VAT												
All	0.60	0.001	0.47	0.01	0.45	0.02	-0.46	0.02	0.21	NS	0.61	0.0006
Obese	0.38	NS	0.42	NS	0.34	NS	-0.35	NS	0.27	NS	0.63	0.02
Controls	0.21	NS	-0.07	NS	-0.15	NS	0.15	NS	-0.21	NS	-0.07	NS
SAT												
All	0.57	0.002	0.39	0.045	0.41	0.03	-0.42	0.03	0.31	NS	0.74	<0.0001
Obese	0.28	NS	0.10	NS	0.07	NS	-0.08	NS	0.34	NS	0.78	0.002
Controls	0.22	NS	0.10	NS	0.06	NS	-0.06	NS	-0.08	NS	0.30	NS
Free testosterone												
All	0.38	NS	0.24	NS	0.27	NS	-0.26	NS	0.46	0.02	0.25	NS
Obese	0.38	NS	0.11	NS	0.13	NS	-0.10	NS	0.58	0.03	0.24	NS
Controls	0.45	NS	0.65	0.04	0.81	0.005	-0.81	0.005	0.42	NS	0.31	NS
SHBG												
All	-0.62	0.001	-0.47	0.02	-0.47	0.02	0.48	0.02	-0.40	0.05	-0.49	0.01
Obese	-0.41	NS	-0.29	NS	-0.17	NS	0.17	NS	-0.47	0.09	-0.64	0.01
Controls	-0.61	0.06	-0.60	0.07	-0.67	0.03	0.67	0.03	-0.44	NS	-0.03	NS
25-OHD												
All	-0.04	NS	0.28	NS	0.25	NS	-0.25	NS	-0.12	NS	-0.26	NS
Obese	0.03	NS	0.47	NS	0.45	NS	-0.46	NS	-0.07	NS	-0.33	NS
Controls	-0.36	NS	-0.22	NS	-0.45	NS	0.45	NS	-0.36	NS	-0.25	NS
PTH												
All	0.07	NS	-0.10	NS	-0.11	NS	0.11	NS	0.18	NS	0.18	NS
Obese	-0.09	NS	-0.58	0.03	-0.60	0.02	0.61	0.02	-0.09	NS	-0.04	NS
Controls	-0.25	NS	0.36	NS	0.44	NS	-0.44	NS	0.33	NS	0.36	NS
PTH/25-OHD												
All	0.14	NS	-0.28	NS	-0.22	NS	0.22	NS	0.13	NS	0.30	NS
Obese	0.14	NS	-0.72	0.008	-0.63	0.03	0.65	0.02	-0.10	NS	0.31	NS
Controls	-0.07	NS	0.40	NS	0.36	NS	-0.35	NS	0.31	NS	0.33	NS

*Log converted values used to approximate a normal distribution and to run Pearson correlations for the group as a whole and for obese girls.

For controls, log conversion did not result in a normal distribution for most variables, and Spearman correlations were used instead.

AUC, area under the curve; HOMA-IR, homeostasis model of insulin resistance; QUICKI, quantitative insulin sensitivity check index; CIR, corrected insulin response; hsCRP, high-sensitivity C-reactive protein; BMI-SDS, body mass index standard deviation score; NS, not significant; AUC, area under the curve; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SHBG, sex hormone-binding globulin; PTH, parathyroid hormone; 25-OHD, 25 hydroxy-vitamin D. Significant associations are represented in bold font.

performed stepwise regression model with (1) PTH/25-OHD, (2) free testosterone or SHBG, and (3) BMI-SDS or VAT entered into the model. When the model included PTH/25-OHD, free testosterone, and BMI-SDS, the ratio of PTH/25-OHD was a significant negative determinant of fasting insulin/glucose and HOMA-IR, and a positive determinant of QUICKI and hsCRP, whereas free testosterone was a positive determinant of CIR (Table 3). When we replaced free testosterone with SHBG, associations of BMI-SDS and PTH/25-OHD with fasting insulin/glucose, HOMA-IR, QUICKI, and hsCRP did not change. SHBG replaced free testosterone as a negative determinant of CIR.

When PTH/25-OHD, free testosterone, and VAT were entered into the model, VAT predicted all parameters except CIR, which was predicted by free testosterone (22% of the variability explained). VAT alone predicted HOMA-IR and QUICKI (31% and 32% of the variability explained), and with PTH/25-OHD accounted for 48% of the variability in

fasting insulin/glucose and 64% of the variability of hsCRP. VAT and free testosterone explained 49% of the variability in insulin AUC. When we replaced free testosterone with SHBG, our findings did not change, except that SHBG (unlike free testosterone) did not independently predict any parameter.

Discussion

After controlling for BMI or VAT, we demonstrated positive associations of PTH/25-OHD levels with hsCRP and inverse associations with measures of insulin resistance in a group of obese and normal weight adolescent girls. Free testosterone was an independent predictor of the corrected insulin response.

Contrary to our expectations, we did not find lower 25-OHD levels in obese versus normal weight groups. There were no differences between groups for season of sampling

TABLE 3. STEPWISE REGRESSION MODEL TO DETERMINE INDEPENDENT PREDICTORS OF INSULIN PARAMETERS AND hsCRP (BMI-SDS, FREE TESTOSTERONE, AND PTH/25-OHD ENTERED INTO THE MODEL)

	Parameter estimate	F ratio	P value	R ²	Cumulative R ²
Insulin AUC ^a					
BMI-SDS	0.074	9.8	0.006	0.35	0.35
Fasting insulin/glucose ^a					
BMI-SDS	0.097	17.0	0.0007	0.33	0.56
PTH/25-OHD	-0.053	8.9	0.008	0.23	
HOMA-IR ^a					
BMI-SDS	0.099	14.6	0.001	0.34	0.50
PTH/25-OHD	-0.045	5.3	0.03	0.16	
QUICKI ^a					
BMI-SDS	-0.015	15.1	0.001	0.35	0.51
PTH/25-OHD	0.007	5.3	0.03	0.16	
CIR ^a					
Free testosterone	0.569	45.6	0.03	0.24	0.24
hsCRP ^a					
BMI-SDS	0.246	40.4	<0.0001	0.67	0.75
PTH/25-OHD	0.074	5.9	0.03	0.08	

^aLog converted values.

hsCRP, high-sensitivity C-reactive protein; BMI-SDS, body mass index standard deviation score; PTH, parathyroid hormone; 25-OHD, 25 hydroxyvitamin D; AUC, area under the curve; HOMA-IR, homeostasis model of insulin resistance; CIR, corrected insulin response.

or the proportion of subjects on calcium and vitamin D supplements. Of note, our obese and normal weight subjects were tightly matched for maturity, race, and ethnicity at study entry. Although it is difficult to speculate on reasons for the lack of difference in 25-OHD levels between the two groups, it is possible that previously reported differences may relate to control populations being less tightly matched for maturity, race, and ethnicity than in this study.

On correlation analysis and in contrast to our initial hypothesis, 25-OHD levels were not associated with any measure of glucose homeostasis within obese girls, normal weight girls, or the groups taken together, whereas PTH levels and PTH/25-OHD were positively associated with insulin sensitivity in obese girls. Although our sample size is relatively small, some larger studies have similarly reported a lack of association between 25-OHD and glucose homeostasis,^{5,14} arguing instead that apparent relationships are confounded by body fat, which demonstrates a strong inverse association with circulating 25-OHD.^{13,14} Of note, associations of the ratio of PTH/25-OHD^{17,23} with insulin measures were stronger than those of PTH alone within obese girls, suggesting a possibly additional, albeit minor, role of lower 25-OHD levels in driving these associations. Thus, further research is needed to investigate associations between 25-OHD and insulin sensitivity independent of fat stores across the weight spectrum.

A possible explanation for the positive association of PTH or PTH/25-OHD with surrogate measures of insulin sensitivity such as QUICKI (and inverse associations with measures of insulin resistance such as HOMA-IR and fasting glucose/insulin) is the stimulation of 1 α -hydroxylation by

PTH. *In vitro* studies demonstrate an important role for 1,25(OH)₂D in β -cell function. 1,25(OH)₂D-deficient rats have decreased insulin synthesis and release in islet cells, which is restored following 1,25(OH)₂D treatment.^{18,19} Moreover, 1,25(OH)₂D downregulates inflammatory cytokine expression in monocytes from patients with type 2 diabetes,²⁰ and addition of 1,25(OH)₂D to β -cell culture protects against cytokine-induced apoptosis.^{21,22} These data support a physiologic role for 1,25(OH)₂D in β -cell preservation and function. Increased PTH may positively affect insulin sensitivity by stimulating production of 1,25(OH)₂D. Alternatively, the observed inverse relationship between PTH or PTH/25-OHD with insulin resistance measures in the current study may reflect PTH-suppressive effects of insulin. Elevated insulin, particularly during euglycemic-hyperinsulinemic clamp conditions, decreases PTH independently of serum calcium,²⁹⁻³¹ although it is unclear whether this effect is sustained with prolonged hyperinsulinemia.^{29,31} One study has reported higher rates of hyperparathyroidism in adults after bariatric procedures, even after accounting for vitamin D levels³²; such procedures are associated with significant reductions in insulin levels. Effects of insulin on PTH merit further investigation.

It is also unclear why PTH and PTH/25-OHD were strongly associated with measures of insulin sensitivity in obese girls but not in matched controls. The lean controls in our cohort, on average, had normal insulin sensitivity, and it is possible that PTH may not play an important physiologic role in glucose homeostasis in the context of normal insulin. Interestingly, after controlling for VAT (strong and important determinant of insulin resistance), associations of PTH/25-OHD with some insulin measures were lost, suggesting that visceral fat may "trump" effects of PTH/25-OHD on insulin.

Studies have variously reported a decrease³³ or no change³⁴ in inflammatory markers following administration of vitamin D. The lack of association of vitamin D levels with hsCRP, a marker of chronic inflammation and cardiovascular risk, is consistent with some prior reports.^{34,35} In addition, a recent study has reported a positive association of PTH with hsCRP in obese adolescents after controlling for vitamin D levels,⁵ consistent with our data. PTH has also been reported to be an independent predictor of systolic (SBP) and diastolic blood pressure (DBP), but not other components of the metabolic syndrome, in morbidly obese adults.³⁶

The association between free testosterone and insulin resistance in the lean cohort (on correlation analysis) and the combined cohort (on regression modeling) is a relatively novel finding in adolescent girls without phenotypic features of polycystic ovary syndrome (PCOS). In female obesity and PCOS, associations between hyperinsulinism, elevated androgen, and decreased SHBG are well known,^{37,38} but the relevance of androgen levels to glucose homeostasis in lean girls without PCOS has not been investigated comprehensively. Lean hyperandrogenic young women have higher measures of insulin resistance than lean normoandrogenic women, and it is possible that positive associations of androgens with measures of insulin resistance exist even when androgens are in a normal range.³⁹ Our findings are consistent with data from hyperinsulinemic-euglycemic clamp studies that demonstrate increased insulin resistance following testosterone administration in female-to-male transsexuals.⁴ Interestingly, in rats, imprinting of the female

offspring with testosterone leads to insulin resistance and changes in body fat distribution in adult life.⁴⁰ The disparate findings between associations of free testosterone with indices of insulin sensitivity in the obese versus control groups are difficult to interpret. This may be an effect consequent to small sample size or related to the spread of data points within groups, because a tight clustering of data points may mask significant associations.

The current study has certain limitations. First, the cross-sectional design precludes assumptions about causality. Thus, we cannot be sure about the directionality of effect between PTH or PTH/25-OHD and measures of insulin sensitivity and cannot exclude all possible confounders. However, we did control for measures of regional adiposity, and associations of PTH/25-OHD with measures of insulin sensitivity remained significant after controlling for BMI-SDS, but were partly abrogated after controlling for VAT. Importantly, PTH/25-OHD was a positive determinant of hsCRP, even after controlling for covariates, including VAT. Unfortunately, measures of 1,25(OH)₂D are not available in the current cohort to assess potential associations with PTH and with insulin sensitivity, nor do we have magnesium or phosphorus levels on our subjects. Of note, although the assay we used for testosterone for this study has a low limit of detection, a more reliable assay for testosterone is high-performance liquid chromatography tandem mass spectrometry. Future studies merit the use of this technique to assess testosterone levels in this population. Additionally, this was a relatively small sample, and the results will be important to confirm in a larger group of subjects. However, this is a very well-characterized and matched group of obese and normal weight controls, and the availability of detailed measures of regional body composition, measures of insulin sensitivity and resistance, and cardiovascular risk markers in these girls is a strength of the study.

In summary, our data demonstrate a positive association of PTH/25-OHD with hsCRP and several insulin measures after controlling for possible confounders. In addition, free testosterone is an independent determinant of the corrected insulin response, an integrated measure of insulin secretion following an oral glucose load. Further investigation is required to clarify the importance of these novel findings and to define mechanisms underlying a possible independent role for PTH in mediating cardiovascular risk and insulin resistance and sensitivity.

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Author Disclosure Statement

The authors have no conflicts of interest to disclose.

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