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ORIGINAL ARTICLE

Association of OPG–RANKL ratio with left ventricular hypertrophy and geometric remodeling in male overweight/obese youths

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Abstract

Purpose Receptor activator of nuclear factor kappa B ligand/receptor activator of nuclear factor kappa B/osteoprotegerin (RANKL/RANK/OPG) axis has been hypothesized as a potential mediator of left ventricular hypertrophy (LVH). The aim of the study was to assess whether circulating concentrations of RANKL, RANK, and OPG were associated with early signs of morphological cardiac changes in overweight/obese youths.

Methods We determined serum levels of RANKL, RANK and OPG by enzyme-linked immunosorbent assays in 188 overweight/obese children and adolescents. LV mass index (LVMI) and relative wall thickness (RWT) were estimated using M-mode echocardiography.

Results OPG and RANKL levels were higher among girls than among boys [1.73 (1.64–1.86) and 3.28 (1.90–6.37) pmol/L, respectively, vs. 1.69 (1.59–1.82) and 2.12 (1.52–3.80) pmol/L; $p = 0.02$ and $p = 0.0001$, respectively], but the OPG/RANKL ratio was lower [0.52 (0.26–0.88) vs 0.77 (0.44–1.11); $p = 0.001$]. In gender-specific multivariate linear regression, OPG/RANKL ratio was associated with LVMI and RWT in boys but not in girls. In multiple logistic regression, after adjustment for clinical variables, OPG/RANKL ratio was associated with concentric remodeling, eccentric and concentric LVH in boys but not in girls.

Conclusion OPG/RANKL ratio is independently associated with LVH and patterns of LV structural remodeling in male overweight/obese children and adolescents.

Keywords Pediatric obesity · Left ventricular hypertrophy · Osteoprotegerin · Receptor activator of nuclear factor kappa B · Receptor activator of nuclear factor kappa B ligand

Introduction

Over the last few decades, obesity has reached epidemic proportions in children and adolescents worldwide. Obesity is associated with insulin resistance, dyslipidemia, elevated blood pressure (BP), and inflammation, which are linked to an increased risk of end organ damage such as left ventricular hypertrophy (LVH) [1].

While in adults LVH has been associated with complications such as myocardial infarction, cerebrovascular events, congestive heart failure, and death [2], this form of abnormal cardiac geometry is usually asymptomatic in youths [1].

However, prospective longitudinal studies have demonstrated that cardiovascular disease (CVD) risk factors present in childhood persist, or track, into adulthood, leading to greater risk of CV events over time [3, 4]. While traditionally thought to be a consequence of increased afterload in hypertensive subjects, LVH is now known to be independently

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associated with measures of adiposity [1, 5]. Contemporary research suggests that there are multiple hemodynamic and non-hemodynamic factors to explain the role of obesity on this pathological remodeling of the heart [1]. Recently, the osteoprotegerin (OPG)/receptor activator of nuclear factor kappa B (RANK)/receptor activator of nuclear factor kappa B ligand (RANKL) axis has been hypothesized as a potential mediator of LVH [6–9]. RANKL is a member of the tumor necrosis factor superfamily ligands discovered in 1997 [10]. Earlier studies have shown that RANKL, via binding to its receptor, RANK, is involved in the regulation of genesis of osteoclasts and antigen presentation by dendritic cells. OPG, as a decoy receptor for RANKL, can weaken or neutralize the biological effects of the latter by binding to it, and thus, it is a negative regulator of RANKL/RANK signaling pathway. The OPG/RANK/RANKL system has a key role in the regulation of activation, differentiation and survival of osteoclasts, and it is necessary for pathophysiology of bone remodeling [11]. Recent experimental, clinical, and epidemiological data have also implicated this system in cardiometabolic diseases [12].

Increased plasma OPG level is an important symbol of RANKL/RANK/OPG system activation. OPG has been associated with dyslipidemia, insulin resistance, endothelial damage, atherosclerosis, coronary artery disease, and adverse cardiovascular outcomes as well as with oversize of LV and increase of the wall thickness [6, 7, 13–17]. In a population-based cohort of more than 2700 adults, OPG was independently associated with indices of LV function in both genders and with indices of LV structure in male but not in female subjects [6]. A subsequent study showed that OPG was independently associated with LV mass in hypertensive African–American men and women [7]. Moreover, animal studies have shown that activation of RANKL/RANK/OPG system is an important pathogenic factor for ventricular remodeling after either myocardial infarction or inflammatory cardiomyopathy [8, 9]. Overall, these studies suggest an involvement of OPG in the pathogenesis of LVH. Nonetheless, the role of OPG in LVH in overweight/obese subjects remains poorly investigated and no study so far has investigated the association of RANKL/RANK/OPG system with LVH and LV structural remodeling in obese youths. Thus, in the present cross-sectional study we aimed to assess whether circulating OPG, RANK, and RANKL concentrations and OPG/RANKL ratio are associated with LVH and patterns of LV geometric remodeling in overweight/obese children and adolescents.

Methods

Study population

A total of 188 overweight/obese children and adolescents (mean age ± standard deviation, 10.4 ± 3.0; 104 boys, and 84 girls) were included in the study. They were consecutively enrolled at the outpatient clinics of the Department of Pediatrics, Sapienza University of Rome, Italy. Exclusion criteria were the presence of renal disease; type 1 or 2 diabetes; any condition known to influence body composition, insulin action, or insulin secretion (e.g., glucocorticoid therapy, hypothyroidism, and Cushing's disease); a history of pre-existing heart disease; any laboratory or clinical evidence of chronic liver disease; and history of alcohol consumption and smoking (where appropriate).

All study participants underwent physical examination including measurements of weight and standing height (from which body mass index (BMI) was calculated), waist circumference (WC), systolic and diastolic BP, and determination of the stage of puberty, as previously reported [18]. The degree of obesity was quantified by Cole's least mean square method, which normalizes the skewed distribution of BMI and expresses BMI as standard deviation score (SDS) [19]. This measure gives age- and gender-specific estimates of the distribution median, the coefficient of variation, and the degree of skew by a maximum-likelihood fitting technique. Systolic and diastolic BP were measured twice at the right arm after a 10 min rest in the supine position using an automated oscillatory system (Dinamap Vital Signs Monitor, Model 1846 SX; Criticon Incorporated, Tampa, FL, USA).

The study was approved by the Hospital Ethics Committee, and informed consent was obtained from subjects' parents before assessment.

Laboratory methods

Blood samples were taken from each subject, after an overnight fast, for estimation of glucose, insulin, total cholesterol, high-density lipoprotein cholesterol, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). An aliquot of serum for measurement of OPG, RANK, and RANKL was stored at − 80° until use. Estimates of insulin sensitivity were calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), defined by fasting insulin and fasting glucose. All analyses were conducted by COBAS 6000 (Roche Diagnostics). While insulin concentrations were measured on cobas 601 module (Electrochemiluminescence Technology, Roche Diagnostics), the remaining analytes were measured on cobas 501 clinical chemistry module (Photometric Technology). OPG, RANK, and RANKL were measured by commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions. OPG and total RANK concentrations were measured using SEA108Hu and SEC57Hu kits, respectively (Cloud-Clone Corp, Houston, TX, USA). For both assays, intra- and interassay coefficients of variation were less than 10 and 12%, respectively. Total RANKL concentrations were measured using Human

sRANKL Kit (Biovendor, Brno, Czech Republic). Intra- and interassay coefficients of variation were less than 11.5 and 12.8%, respectively.

Echocardiography

Echocardiography was performed with a commercially available echocardiographic system equipped with a 4 MHz phased array transducer (SONOS 5500, Phillips, Andover, Massachusetts, USA), as previously reported [20]. Guided by two-dimensional echocardiography, standard M-mode recordings of the LV measurements including LV dimension at end diastole (LVDd), LV posterior wall thickness at end diastole (LVPWd), interventricular septal thickness at end diastole (IVSd), LV dimension at end systole (LVDs), LV posterior wall thickness at end systole (LVPWs), and interventricular septal thickness at end systole (IVSs) were obtained according to the American Society of Echocardiography (ASE) [21]. Relative wall thickness (RWT) was calculated using the following formula: IVSd + LVPWd/LVDd. LV mass was calculated by the ASE method, normalized for body height in meters to the allometric power of 2.7 (which linearizes the relation between LV mass and body growth and identifies the impact of excess body weight) and expressed as an index (LVMI).

Definitions

LVH was defined as LVMI exceeding the 95th percentile for gender and chronological age according to Khoury et al. [22]. High levels of RWT were defined using a cut-off point of 0.375 [23]. LV geometry was divided as: normal geometry (normal LVMI and normal RWT), eccentric LVH (LVH but normal RWT), LV concentric remodeling (increased RWT but normal LVMI), and concentric LVH (increased RWT and LVH) [23].

Statistical analysis

Statistical analyses were performed using the SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). Shapiro–Wilk test was used to verify the normality of distribution of continuous variables. When data were not normally distributed, logarithmic transformation was performed. Data are reported as means and standard deviations for normally distributed variables, or as median and (25th–75th) for non-normally distributed variables. Differences between genders were evaluated by *t* test or Mann–Whitney *U* test, as appropriate. Proportions were compared by the χ^2 or Fisher exact. Pearson (normal distribution) and Spearman (non-normal distribution) correlation analysis were used to assess the correlations between the continuous parameters. As circulating levels of OPG and RANKL were significantly higher while the OPG/RANKL ratio was lower in girls than in boys, the associations between OPG, RANK, and RANKL concentrations and indices of LV structure such as LVMI and RWT were determined separately for boys and for girls using multivariate linear regression models. To determine the risk factors of LVH, multivariate logistic regression (backward method) analysis was employed. In both linear and logistic multivariate regression models, only variables significantly associated with the respective dependent variables were included. A p value of less than 0.05 was considered statistically significant.

Results

Clinical and laboratory characteristics of the study population

The clinical and biochemical characteristics of overweight/obese children and adolescents according to gender are presented in Table 1. Males were on average older than females and had higher values of WC. However, no differences between males and females were observed in BMI-SDS score, waist-to-height ratio, and waist-to-hip ratio. Also, there were no differences between the two groups with respect to systolic and diastolic BP, lipid profile, AST, ALT, fasting glucose, insulin concentrations and HOMA-IR values, as well as indices of LV structure and patterns of LV geometric remodeling. OPG and RANKL levels were higher among girls than among boys [1.73 (1.64–1.86) and 3.28 (1.90–6.37) pmol/L, respectively, vs 1.69 (1.59–1.82) and 2.12 (1.52–3.80) pmol/L; $p = 0.02$ and $p = 0.0001$, respectively], but the OPG/RANKL ratio was lower [0.52 (0.26–0.88) vs 0.77 (0.44–1.11); $p = 0.001$]. No differences between the two groups were observed with regard to RANK concentrations.

OPG, RANK, and RANKL levels and OPG/RANKL ratio in relation to clinical and biochemical parameters, and indices of LV structure

In boys, RANKL was negatively correlated with LVMI ($p < 0.001$) and RWT ($p < 0.01$), while OPG and OPG/RANKL ratio were positively correlated with LVMI ($p < 0.01$ and $p < 0.001$, respectively) and RWT ($p < 0.01$ and $p < 0.001$, respectively) (Table 2). No correlation was observed in boys between RANK levels and indices of LV structure. Among other variables, fasting glucose was positively correlated with RANKL concentrations but negatively correlated with OPG/RANKL ratio. RANK values were associated with fasting insulin and diastolic BP. In girls, no associations were demonstrated between OPG, RANK, and RANKL levels and OPG/RANKL ratio and indices of LV

Table 1 Clinical and laboratory characteristics of the study population

	All subjects ($n=188$)	Males ($n=104$)	Females ($n=84$)	p
Anthropometric variables				
Age (years)	10.4 (3.0)	10.9 (3.0)	9.7 (2.9)	0.007
Age < 10 years, *n* (%)	82 (43.6)	34 (32.7)	48 (57.1)	0.0008
Age ≥ 10 years, *n* (%)	106 (56.4)	70 (67.3)	36 (42.9)	0.0008
BMI-SDS	2.0 (0.5)	2.0 (1.8–2.3)	1.9 (1.7–2.2)	0.22
Overweight, *n* (%)	95 (50.5)	48 (46.1)	47 (56.0)	0.18
Obese, *n* (%)	93 (49.5)	56 (53.8)	37 (44.0)	0.18
Waist circumference (cm)	86.6 (11.9)	88.9 (11.9)	83.6 (11.4)	0.003
Waist-to-height ratio[a]	0.59 (0.06)	0.60 (0.06)	0.59 (0.05)	0.49
Waist-to-hip ratio[a]	0.92 (0.11)	0.92 (0.13)	0.92 (0.06)	0.37
Metabolic variables				
Total cholesterol (mg/dL)	159 (33)	160 (33)	158 (32)	0.79
HDL-C (mg/dL)	50 (13)	49 (14)	51 (12)	0.28
Triglycerides (mg/dL)	68 (50–101)	71 (51–106)	61 (49–99)	0.33
Fasting glucose (mg/dL)	83 (6)	83 (6)	82 (7)	0.21
Fasting insulin (μU/mL)	11 (8–16)	11 (7–16)	11 (8–17)	0.55
HOMA-IR	2.4 (1.6–3.8)	2.5 (1.5–3.8)	2.4 (1.8–3.8)	0.63
AST (U/L)	23 (20–26)	23 (20–26)	22 (19–26)	0.26
ALT (U/L)	18 (15–26)	18 (15–31)	18 (14–24)	0.14
Cardiac variables				
Systolic blood pressure (mmHg)	110 (14)	111 (13)	108 (15)	0.13
Diastolic blood pressure (mmHg)	65 (10)	65 (10)	64 (10)	0.42
LV mass index (g/height$^{2.7}$)	38 (11)	39 (12)	36 (11)	0.15
RWT (mm)	0.36 (0.08)	0.36 (0.08)	0.36 (0.09)	0.92
LV remodeling patterns				
Eccentric LVH, *n* (%)	70 (37.2)	36 (34.6)	34 (40.5)	0.45
Concentric remodeling, *n* (%)	68 (36.2)	39 (37.5)	29 (34.5)	0.76
Concentric LVH, *n* (%)	41 (21.8)	25 (24.0)	16 (19.0)	0.48
OPG/RANK/RANKL				
OPG (pmol/L)	1.71 (1.61–1.84)	1.69 (1.59–1.82)	1.73 (1.64–1.86)	0.02
RANK (ng/L)	2.41 (1.63–3.60)	2.33 (1.63–3.33)	2.42 (1.63–3.67)	0.55
RANKL (pmol/L)	2.61 (1.62–4.55)	2.12 (1.52–3.80)	3.28 (1.90–6.37)	0.0001
OPG/RANKL ratio	0.65 (0.0.36–1.07)	0.77 (0.44–1.11)	0.52 (0.26–0.88)	0.001

BMI-SDS body mass index-standard deviation score, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LV* left ventricular, *RWT* relative wall thickness, *LVH* left ventricular hypertrophy, *OPG* osteoprotegerin, *RANK* receptor activator of nuclear factor kappa B, *RANKL* receptor activator of nuclear factor kappa B ligand

[a]log10-transformed

structure (Table S1). Among other variables, RANKL was negatively associated with age and diastolic BP, while OPG/RANKL ratio was positively correlated with diastolic BP.

Multivariate regression analysis of the association of OPG/RANKL ratio and indices of LV structure and LV remodeling patterns

In gender-specific multivariate linear regression analyses, OPG/RANKL ratio was associated with LVMI and RWT ($p<0.0001$ and $p<0.0001$, respectively) in boys after adjustment for age and Tanner stage (Table 3A, B). LVMI was also associated with BMI-SDS ($p<0.0001$) (Table 3A), while RWT was associated with BMI-SDS and triglycerides ($p=0.01$ and $p=0.04$, respectively) (Table 3B). OPG/RANKL ratio explained 18 and 13% of the variance of LVMI and RWT, respectively. The two models shown in Table 3A, B explained the 33 and 20% of the total variance (R^2 adjusted) of LVMI and RWT, respectively, after adjusting for age and Tanner stage. In girls, LVMI was associated with fasting glucose ($p=0.01$), while none of the variables were associated with RWT (Tables S2A and S2B).

Table 2 Correlations between OPG, RANK, and RANKL levels and OPG/RANKL ratio and clinical and biochemical parameters, and indices of LV structure in boys

	OPG (pmol/L)	RANK (ng/L)	RANKL (pmol/L)	OPG/RANKL ratio	LVMI (g/height$^{2.7}$)	RWT (mm)
Age (years)	−0.1	0.07	−0.05	0.03	−0.17	−0.02
BMI-SDS	0.07	−0.04	−0.01	0.03	0.38****	0.29**
Waist circumference (cm)	−0.005	0.08	−0.03	0.03	−0.02	0.09
Total cholesterol (mg/dL)	0.13	0.05	−0.005	0.006	0.08	0.08
HDL-C (mg/dL)	0.03	−0.10	−0.04	0.03	−0.01	−0.14
Triglycerides (mg/dL)	0.01	0.08	0.05	−0.03	0.04	0.19*
Fasting glucose (mg/dL)	−0.08	−0.13	0.20*	−0.20*	−0.08	−0.01
Fasting insulin (μU/mL)	0.12	0.21*	−0.13	0.15	0.10	0.17
HOMA-IR	0.11	0.19	−0.10	0.12	0.07	0.17
AST (U/L)	0.12	0.14	−0.11	0.11	0.19	0.03
ALT (U/L)	0.04	0.09	−0.01	−0.002	0.09	0.03
Systolic BP (mmHg)	−0.12	−0.12	0.09	−0.12	0.10	0.08
Diastolic BP (mmHg)	0.06	0.20*	−0.07	0.06	0.05	0.13
OPG (pmol/L)	–	0.53****	−0.21*	0.35***	0.28**	0.28**
RANK (ng/L)	0.53****	–	−0.28**	0.31**	0.10	0.10
RANKL (pmol/L)	−0.21*	−0.28**	–	−0.99****	−0.37***	−0.31**
OPG/RANKL ratio	0.35***	0.31**	−0.99****	–	0.37***	0.36***

OPG osteoprotegerin, *RANK* receptor activator of nuclear factor kappa B, *RANKL* receptor activator of nuclear factor kappa B ligand, *LV* left ventricular, *LVMI* LV mass index, *RWT* relative wall thickness, *BMI-SDS* body mass index-standard deviation score, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *BP* blood pressure

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

Table 3 Multivariate linear regression analysis for dependent variable (A) LVMI[a] and (B) RWT[a] in boys

	β coefficient ± standard error[b]	95% confidence intervals	p value	$R^2_{partial}$
(A)				
OPG/RANKL ratio	0.11 ± 0.02	0.07–0.15	< 0.0001	0.18
BMI-SDS	0.10 ± 0.02	0.06–0.14	< 0.0001	0.15
(B)				
OPG/RANKL ratio	0.05 ± 0.01	0.02–0.07	< 0.0001	0.13
BMI-SDS	0.03 ± 0.01	0.006–0.05	0.01	0.04
Triglycerides	0.0002 ± 0.0001	0.00001–0.0004	0.04	0.03

Independent variables: BMI-SDS, triglycerides, OPG, RANKL, OPG/RANKL. $R^2_{partial}$ indicates the increased percentage of the variation explained when each variable is added to the model

[a]log10-transformed

[b]Adjusted for age and Tanner stage

To further investigate the association of OPG/RANKL ratio with LVH and LV remodeling patterns, we performed a multivariate logistic regression analysis, using the backward elimination method. In boys (Table 4A–C), independent risk factors for eccentric LVH were OPG/RANKL ratio [Odds ratio (OR) = 6.2, $p = 0.0002$]; for concentric remodeling were OPG/RANKL ratio (OR = 4.1, $p = 0.002$) and triglycerides (OR = 1.01, $p = 0.01$); and for concentric LVH were OPG/RANKL ratio (OR = 6.6, $p = 0.0003$) and BMI-SDS (OR = 2.5, $p = 0.04$). In girls, we found that only high glucose levels represented a risk factor for concentric remodeling (OR = 18.3, $p = 0.03$) (data not shown).

Discussion

The main finding from our study is that circulating OPG, RANKL and OPG/RANKL ratio are associated with LVMI and RWT in male overweight/obese children and adolescents. OPG levels and OPG/RANKL ratio displayed positive

Table 4 Multivariate logistic regression for (A) eccentric LVH, (B) concentric remodeling and (C) concentric LVH in boys

	Odds ratio[a]	95% confidence intervals	*p* value
(A)			
OPG/RANKL ratio	6.2	2.3–16.3	0.0002
(B)			
OPG/RANKL ratio	4.1	1.7–10.1	0.002
Triglycerides	1.01	1.00–1.02	0.01
(C)			
OPG/RANKL ratio	6.6	2.4–18.2	0.0003
BMI-SDS	2.5	1.0–6.3	0.04

Independent variables: BMI-SDS, triglycerides, fasting insulin, OPG, RANKL, OPG/RANKL

[a]Adjusted for age and Tanner stage

associations, while RANKL concentrations showed inverse associations with indices of LV structure. Additionally, in these subjects, OPG/RANKL ratio predicted eccentric LVH, concentric remodeling and concentric LVH, independent of traditional CVD risk factors. To the best of our knowledge, this is the first study to report a close association between OPG/RANKL levels and cardiac structural and geometric changes in a pediatric population.

Recent epidemiological and clinical evidence from human studies suggest that the OPG/RANK/RANKL axis not only plays an important role in bone metabolism, but also has great importance in the pathogenesis of many cardiovascular diseases. Studies in humans have demonstrated that elevated levels of serum OPG are associated with an increase of intima-media thickness as well as with a reduction of brachial flow-mediated vasodilation, markers of early atherosclerosis [15, 16]. Several investigations carried out in large populations have also demonstrated that an increased release of OPG is associated with increased cardiovascular risk [17]. In particular, increased serum OPG has been associated with greater risk of myocardial infarction, ischemic stroke, total mortality, and death due to ischemic heart disease and nonvascular causes, after adjustment for several cardiovascular risk factors. Interestingly, polymorphisms in the OPG gene also seem to be linked to CVD [24]. Moreover, clinical studies in adults have demonstrated that plasma OPG levels are correlated with LVH [6, 7].

Data regarding the clinical cardiometabolic correlates of RANKL are sparse and contradictory. In the prospective population-based study conducted in Brunico, Italy, levels of RANKL did not differ between genders and were not related to age, menopausal status, lifestyle characteristics, smoking or diabetes [25]. In the European Prospective Investigation into Cancer and Nutrition study, soluble RANKL levels were significantly associated with age, lipid parameters, BMI, and blood pressure in men, and C reactive protein in women [26]. Although there was an association between RANKL and future coronary events in men, this association was lost when adjusting for other risk factors. In a cohort of 522 white men who underwent coronary angiography, Shoppet et al. demonstrated that RANKL serum levels were significantly lower in patients with coronary artery disease (CAD) compared with those without CAD, but in contrast to OPG serum levels, who were not correlated with the severity of CAD [27]. RANKL levels were not correlated with age, BMI, or the presence of diabetes, arterial hypertension, or hyperlipemia. They were, however, correlated negatively with OPG serum levels. Yet, in a large sample from the general population including more than 3200 participants, Lieb et al. observed higher RANKL levels in women and modest inverse associations of RANKL with age, smoking, diabetes, antihypertensive treatment (as a marker of chronic hypertension), and HDL-C, indicating that RANKL might reflect a more favorable CVD risk profile [28]. Our study, which is the first to examine the relationship between serum RANKL concentrations and LV structural changes, provides support for a role of RANKL on cardiac structure and geometry in obese youths. Indeed, the concurrent changes of OPG and RANKL with an increase of the OPG-to-RANKL ratio in our male obese pediatric population were associated with changes in LV structure and patterns of LV remodeling. The interplay between RANKL and its naturally occurring decoy receptor OPG deserves further consideration. Under normal circumstances, OPG is thought to neutralize RANKL activity by inhibiting RANKL binding to RANK. However, OPG may, at least at low OPG/RANKL ratio, enhance the matrix metalloproteinase (MMP)-inducing effect of RANKL, and at high concentration, have MMP-inducing and chemotactic effects of its own, suggesting a more direct role of OPG in the promotion of CVD [29, 30].

As the design of our study is cross-sectional, we cannot deduce whether the OPG/RANKL ratio is causally linked to an increase in LVMI and RWT or whether it merely represents an epiphenomenon. However, the association between the OPG/RANKL ratio and indices of LV structure persisted in male youths after statistical adjustment for potential confounders. The reason for the lack of associations between OPG/RANKL ratio and indices of LV structure in girls remains unclear. One possible explanation is that the smaller sample size of girls compared to boys did not allow us to demonstrate a statistical relationship. Nonetheless, our findings are in line with other cross-sectional human studies examining the pleiotropic effects of the RANKL/RANK/OPG axis. Published findings of the Rancho Bernardo Study in an osteoporosis cohort of older men and postmenopausal women demonstrated a modulatory effect of both endogenous and exogenous sex hormones on the biologic interaction of OPG, RANKL, and bone [31]. Only men showed a RANKL, RANKL/

OPG ratio–bone mineral density association. Yet, the population-based, multiethnic Dallas Heart Study showed that OPG was independently associated with indices of LV hypertrophy in male but not females subjects [6]. Thus, we believe future studies on the relationship between sex steroids and the RANKL/RANK/OPG system in a large sample of female and male youths may help to understand the differential gender associations between OPG/RANKL ratio and cardiac structural and geometric abnormalities.

Because LVH is usually asymptomatic in children, studies in this age group are important. Indeed, prospective longitudinal studies have demonstrated that CV risk factors present in childhood persist, or track, into adulthood, leading to greater risk of CV events over time [32–34]. In fact, studies from the Bogalusa Heart Study demonstrated that CV risk factors present in childhood are predictive of coronary artery disease in adulthood [35, 36]. Among these risk factors, low density lipoprotein cholesterol (LDL) and body mass index (BMI) measured in childhood were found to predict cIMT in young adults [35, 36]. Moreover, it has been shown that the association between CV risk factors and excessive cardiac growth occurs in children and adolescents [37], and that early life risk factors significantly predict adulthood LVH and LV geometric patterns [38, 39].

Conclusions

Although it remains unclear whether OPG/RANKL is a marker or rather plays a causal role in mediating cardiac injury, OPG/RANKL may be a promising biomarker of LVH and cardiac remodeling in asymptomatic obese male youths. Early identification of children and adolescents with LVH and LV geometric remodeling provides an excellent opportunity for primary prevention at the population level, by instituting simple lifestyle changes, such as weight loss and regular physical exercise that can prevent development of subsequent CVD.

Compliance with ethical standards

Conflict of interest No disclosure for any prior publication or submission. No conflict of interest or role of any sponsor is present in this work. The authors alone are responsible for the content and the writing of the paper.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individuals, parents/guardians of participants included in the study.

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