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Serum IL-1 Receptor Antagonist Concentrations Associate With Unfavorable Metabolic Features in 12-Year-Old Children

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Context: Elevated IL-1 receptor antagonist (IL-1Ra) concentrations are associated with obesity, insulin resistance, and cardiovascular disease (CVD) risk in adults.

Objective: To determine if serum IL-1Ra and high-sensitivity C-reactive protein (hs-CRP) levels are associated with markers of reduced insulin sensitivity (IS) and serum lipids in 12-year-old children.

Design and Participants: Of 191 children (n = 109 girls), 78 were categorized as having had birth weight and length appropriate for gestational age (AGA), 69 were small for gestational age, and 44 were AGA and from preeclamptic pregnancies. Serum markers of low-grade inflammation, IS, and lipids were measured. Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated.

Results: Mean serum IL-1Ra levels did not differ between the sexes or among the gestational categories. Children in the highest IL-1Ra tertile had lower QUICKI, IGF-binding protein-1, SHBG, and high-density lipoprotein cholesterol values; and higher body mass index (BMI), waist circumference to height ratio (WHtR), and serum insulin, hs-CRP, leptin, and triglyceride concentrations than those in the lowest IL-1Ra tertile. Logistic regression analysis showed higher serum hs-CRP and leptin levels, and WHtR were associated with high serum IL-1Ra levels. IL-1Ra concentration could be used to discriminate the children with lowest IS (area under the curve, 0.68; $P < 0.001$); hs-CRP level could not.

Conclusion: Children with the highest IL-1Ra levels had lower IS, higher hs-CRP levels and BMI, and a less favorable lipid profile than those with the lowest IL-1Ra levels, suggesting that high IL-1Ra concentrations may be associated with increased CVD risk in 12-year-old children.

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Freeform/Key Words: adipocytokines, cardiovascular disease risk, dyslipidemia, inflammation, insulin sensitivity, high-sensitivity CRP (hs-CRP)

Adipose tissue is a metabolically active organ that secretes a variety of adipocytokines, which are able to modulate immunological responses, energy homeostasis, adipogenesis, and insulin sensitivity (IS) [1]. Metabolic stress in the white visceral adipose tissue causes chronic subclinical inflammation, which may induce activation of the innate immune system, leading

Abbreviations: AGA, appropriate for gestational age; apoB, apolipoprotein-B; AUC, area under the receiver operating characteristic curve; BMI, body mass index; BMIadj, sex- and adult age–adjusted body mass index; CV, coefficient of variation; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IGFBP-1, IGF binding protein-1; IL-1Ra, IL-1 receptor antagonist; IS, insulin sensitivity; PRE, preeclamptic pregnancy; QUICKI, Quantitative Insulin Sensitivity Check Index; ROC, receiver operating characteristic; SGA, small for gestational age; WHtR, waist circumference to height ratio.

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to dysregulated adipocytokine synthesis, insulin resistance, β-cell dysfunction, and type 2 diabetes [1–4]. Furthermore, proinflammatory cytokines contribute to vascular endothelial dysfunction and, finally, to atherosclerotic cardiovascular disease (CVD) [4, 5].

The IL-1 cytokine family is a critical regulator of the innate immune responses [3, 5, 6]. It consists of 11 members [6], almost all of which are involved in visceral obesity-associated inflammation [3]. The proinflammatory cytokine IL-1β induces secretion of other proinflammatory cytokines, inhibits β-cell function, destroys β-cells, and promotes insulin resistance [3, 4]. Its natural antagonist is the adipose tissue–derived cytokine IL-1 receptor antagonist (IL-1Ra) [7], which acts by blocking the binding site of IL-1β at the IL-1 receptor [6]. IL-1Ra synthesis is induced by many inflammatory factors, including the members of the IL-1 family [3].

IL-1β induces IL-1Ra production; thus, upregulated IL-1Ra levels are considered to reflect higher IL-1β activity [8] and predispose to insulin resistance and type 2 diabetes [3]. In adults, elevated IL-1Ra concentrations are associated with risk for type 2 diabetes [4, 8–10], the metabolic syndrome and obesity [3], essential hypertension [11] and CVD [4, 8]. Furthermore, increased IL-1Ra concentrations in obesity in childhood are a sign of low-grade inflammation [12]. Moreover, IL-1Ra was considered the most sensitive marker of cytokine response in the prediabetic state in the offspring of patients with type 2 diabetes [9].

The aim of the current study was to determine whether serum IL-1Ra concentrations are associated with other markers of low-grade inflammation, reduced IS, or unfavorable lipid profile in 12-year-old children. Furthermore, we investigated whether IL-1Ra concentrations are associated with low birth weight or exposure to maternal preeclampsia, which are considered to independently predispose to later metabolic and CVDs [13–15]. Finally, we compared concentrations of IL-1Ra with high-sensitivity C-reactive protein (hs-CRP) in terms of detecting reduced IS and unfavorable lipid profile.

1. Material and Methods

A. Definitions

Preeclampsia was defined as the development of hypertension and proteinuria (>300 mg of urinary protein in 24 hours) after 20 weeks of gestation [16]. Hypertension was defined as blood pressure >140/90 mm Hg or a rise of ≥30/15 mm Hg from the baseline level confirmed by two measurements at least 6 hours apart. Birth at or after week 37 and before the 42nd week of gestation was considered full term; birth before the 37th week of gestation (calculated from the beginning of the last menstruation) was considered preterm. Small for gestational age (SGA) was defined as birth weight and/or length >2 SDs below the respective mean for the gestational age and sex. Appropriate for gestational age (AGA) was defined as birth weight and birth length equal to or above −2 SDs and equal to or below +2 SDs of the respective mean for gestational age and sex [17].

B. Subjects

This study population consisted of a cohort of 191 12-year-old children who originally were recruited for a study investigating the metabolic consequences of either being born SGA or being born after a preeclamptic pregnancy (PRE). Of this cohort, 109 were girls, 69 were born SGA, and 44 were born after a PRE as AGA. The median of the gestational ages was 38.0 weeks (range, 28 to 42 weeks). The extremely preterm children born before week 28 of gestation were excluded from the study. None of the participating children was exposed to exogenous glucocorticoids prenatally. All children were born at Kuopio University Hospital, Kuopio, Finland, during 22 months between 1984 and 1986. The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the child and the parents.

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C. Methods

The perinatal data, anthropometric measures, and ambulatory blood pressure values at 12 years age have been described previously for the SGA [18, 19] and PRE children [20, 21]. Pubertal development was classified as Tanner stages according to breast development in girls and genital development in boys. Body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) and sex- and adult age–adjusted BMI (BMIadj; corresponding to the BMI values at the age of 18 years [22]) were calculated. Waist circumference to height ratio (WHtR) was calculated by dividing waist circumference (in centimeters) by height (in centimeters). Perinatal characteristics, anthropometric measures, and pubertal development at the age of 12 years in the whole population are presented in Table 1.

C-1. Laboratory methods

Blood samples were taken in the morning, between 9:00 AM and 10:00 AM, after an overnight fast. An IV cannula was placed in the antecubital vein for blood sampling. After the child had rested for 1 hour in a recumbent position, blood samples were collected through the cannula. Serum specimens were immediately frozen and stored at −70°C until analyzed.

Serum IL-1Ra concentrations were measured by ELISA (human IL-1ra Quantikine ELISA Kit; R&D Systems, Minneapolis, MN). The intra- and interassay coefficients of variation

Table 1. Anthropometric Characteristics of the Study Population at Birth and at Age 12 Years

Variable	All (N = 191)	Girls (n = 109)	Boys (n = 82)	*P* Value[a]
At birth				
Gestational age, wk				0.043
Mean	37.7	38.1	37.2	
Median	38.0	39.0	38.0	
Range	28–42	29–42	28–42	
Weight, g	2773 (2666–2881)	2736 (2586–2886)	2823 (2669–2978)	0.482
Weight (SDS)	−1.13 (−1.33 to −0.94)	−1.27 (−1.55 to −1.00)	−0.95 (−1.21 to −0.69)	0.070
Length, cm	47.3 (46.8–47.8)	47.0 (46.3–47.8)	47.7 (46.9–48.5)	0.319
Length (SDS)	−0.87 (−1.08 to −0.66)	−1.02 (-1.31 to -0.74)	−0.67 (−0.98 to −0.36)	0.091
Age 12 years				
Age, y	12.26 (12.23–12.29)	12.27 (12.22–12.31)	12.26 (12.22–12.29)	0.970
Weight, kg	44.13 (42.61–45.65)	44.35 (42.27–46.43)	43.83 (41.57–46.09)	0.835
BMIadj, kg/m^2	21.44 (20.89–21.99)	20.94 (20.26–21.61)	22.10 (21.21–23.00)	0.060
Height, cm	153.2 (152.1–154.2)	153.9 (152.4–155.4)	152.2 (150.5–153.8)	0.208
Height (SDS)	0.26 (0.12–0.41)	0.16 (−0.04 to 0.36)	0.40 (0.18–0.62)	0.061
WHtR				0.002
Mean	0.43	0.42	0.44	
Median	0.42	0.41	0.43	
Range	0.35–0.63	0.35–0.63	0.36–0.63	
Pubertal development[b] (early or late stage), no.	106/85	38/71	68/14	<0.001[c]
Tanner B/G stage, no.				<0.001[c]
1	40	14	26	
2	66	24	42	
3	50	38	12	
4	27	25	2	
5	8	8	0	

Data are given as mean (95% CI) or, for the variables with skew distributions, as mean, median and range.
Abbreviations: B/G, breast/genital development; SDS, SD score.
[a]Mann-Whitney *U* test for the differences between the girls and boys.
[b]Early stage of puberty: breast or genital development scores, 1–2; late stage of puberty: breast or genital development scores 3–5.
[c]χ^2 test for the differences between the girls and boys.

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(CVs) for IL-1Ra were 3.6% and 5.7%, respectively. Serum hs-CRP concentrations were determined by a photometric immunoturbidimetric method (Konelab 20XT Clinical Chemistry Analyzer; Thermo Fisher Scientific, Vantaa, Finland), and the interassay CVs were 5.0% at 0.78 mg/L and 2.3% at 2.55 mg/L. Serum insulin concentrations were determined by RIA (Phadeseph Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden); the intra- and interassay CVs were 5.3% and 7.6%, respectively. Blood glucose concentrations were analyzed by a glucose oxidase method (Enzyme Electrode; Nova Biomedical, Waltham, MA), and the respective CVs were 3% and 5%. Serum high-density lipoprotein cholesterol (HDL-C) and triglyceride levels were measured enzymatically by an automatic photometric method (Roche Molecular Biochemicals, Mannheim, Germany); the interassay CVs for HDL-C were 4.1% at 0.84 mM and 3.8% at 1.69 mM, and for triglycerides were 3.2% at 1.23 mM and 1.6% at 2.45 mM. Serum IGF-binding protein-1 (IGFBP-1) concentration was analyzed by ELISA (DSL-10-7800 ACTIVE Total IGFBP-1 ELISA; Diagnostic Systems Laboratories, Webster, TX). The intra- and interassay CVs for IGFBP-1 were 2.5% and 6.8%, respectively. Serum SHBG level was measured by the AutoDELFIA SHBG time-resolved fluoroimmunoassay method (Perkin Elmer Life Sciences Wallac, Turku, Finland). The intra- and interassay CVs were 4.0% and 2.6%, respectively. Serum leptin and adiponectin concentrations were analyzed by ELISA (Quantikine DLP00 and Quantikine DRP300; R&D Systems); intra- and interassay CVs for leptin were 3.2% and 2.5% to 4.7%, and for adiponectin were 3.5% and 5.8% to 6.9%. Serum apolipoprotein-B (apoB) level was also analyzed by immunoturbidimetry (Konelab 20XT Clinical Chemistry Analyzer; Thermo Fisher Scientific); the interassay CV was 1.1%. Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as 1/[log (fasting insulin, measured in microunits per milliliter) + log (fasting glucose, measured in milligrams per deciliter)] [23].

D. Statistical Analyses

Data were analyzed using the statistical program SPSS for Macintosh, version 24.0 (IBM, Armonk, NY). All continuous variables were examined for normality with the Kolmogorov-Smirnov test. Because most of the measured variables did not distribute normally, the nonparametric Mann-Whitney *U* test was used for the comparisons between the continuous variables, and the χ^2 test was used for the categorical variables. Analysis of covariance was used to exclude the effects of confounding factors on the results. The Kruskal-Wallis test was used in the comparisons of the lowest, middle and highest IL1-Ra tertiles. Adjusted *P* values (Bonferroni correction) were reported in these analyses. Because of the skew distributions of some variables, the median and range were reported in addition to the mean. Partial correlation coefficients were calculated.

Logistic regression analyses were used to explore independent associating factors with high serum levels of IL-1Ra or high levels of hs-CRP. Receiver operating characteristic (ROC) curve analyses were used to explore whether IL-1Ra or hs-CRP concentrations could be used to identify the children with the lowest QUICKI values. Multivariable regression analyses were performed to evaluate the independent contributions of serum inflammatory markers and BMIadj to IS and lipid variables. In parametric tests, skewed variables were either logarithmically or square-root transformed before testing. $P < 0.05$ was accepted as significant in all analyses. hs-CRP values >10 mg/L (n = 2 boys, n = 1 girl) were excluded from the hs-CRP analyses because of the possibility of acute infections.

2. Results

A. Anthropometric Characteristics and Pubertal Development

At the age of 12 years, the boys had a higher WHtR than the girls ($P < 0.002$), whereas the difference in BMIadj did not reach statistical significance (Table 1). Pubertal development was more advanced in the girls than boys: 87% of the girls and 68% of the boys had pubertal

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signs (Tanner stage for breast development/genital development, ≥2; Table 1). Weight or height did not differ significantly between the girls and boys. The children in the SGA group were shorter and leaner compared with the those in the AGA and PRE groups in terms of height SD scores and BMIadj (SGA vs AGA: −0.19 and 0.63, $P < 0.001$; 20.33 and 22.14 kg/m^2, $P = 0.034$, respectively; and SGA vs PRE: −0.19 and 0.33, $P = 0.022$; 20.33 and 21.94 kg/m^2, $P = 0.032$, respectively, by Bonferroni-adjusted Kruskall-Wallis test). Pubertal development did not differ among the SGA, AGA, or PRE subgroups or between the children born preterm or at full term (data not shown).

B. Inflammation and Insulin Sensitivity Markers and Lipids

The mean concentrations of serum IL1-Ra or hs-CRP did not differ between the girls and boys (Table 2). However, the girls had significantly higher serum insulin, leptin, and triglyceride concentrations, and lower QUICKI, blood glucose, and serum IGFBP-1 values than the boys (Table 2). The sex differences remained essentially similar even after adjustment for pubertal developmental stage and BMIadj (performed by analysis of covariance; data not shown). Serum IL-1Ra, hs-CRP, lipids, or IS markers (*i.e.*, QUICKI, IGFBP-1, adiponectin, SHBG) did not differ among the AGA, SGA, and PRE groups or between the children born at full term or preterm (data not shown).

C. Factors Associated With Higher Serum IL-1Ra Concentrations

The children in the highest IL1-Ra tertile had significantly lower QUICKI, IGFBP-1, and SHBG values, and higher values for BMIadj, WHtR, leptin, and hs-CRP when compared with the children in the lower IL1-Ra tertiles (Table 3). Height SD scores did not differ

Table 2. Biochemical Parameters in the Whole Study Population

Variable	All (N = 191)	Girls (n = 109)	Boys (n = 82)	*P* Value[a]
Serum IL-1Ra, pg/mL				0.610
Mean	285.9	274.4	301.1	
Median	222.3	226.2	219.1	
Range	31.1–1605.8	31.1–1151.2	94.0–1605.8	
Serum hs-CRP, mg/L	n = 187	n = 107	n = 80	0.170
Mean	0.64	0.50	0.82	
Median	0.09	0.09	0.12	
Range	0.09–9.43	0.09–5.52	0.09–9.43	
Serum insulin, mU/L	10.2 (9.6–10.8)	11.1 (10.2–12.1)	8.9 (8.2–9.5)	0.001
Blood glucose, mmol/L	4.3 (4.3–4.4)	4.3 (4.2–4.3)	4.4 (4.3–4.5)	0.022
QUICKI	0.351 (0.348–0.354)	0.347 (0.342–0.352)	0.356 (0.351–0.361)	0.006
Serum IGFBP-1, μg/L	66.1 (61.0–71.3)	60.9 (54.3–67.5)	73.1 (65.0–81.3)	0.022
Serum SHBG, nmol/L	73.6 (68.9–78.3)	69.6 (63.7–75.5)	78.9 (71.4–86.5)	0.097
Serum leptin, μg/L				<0.001
Mean	15.1	18.0	11.2	
Median	9.3	12.3	6.8	
Range	0.3–101.5	0.3–101.5	0.4–58.8	
Serum adiponectin, mg/L	11.0 (10.1–11.8)	11.2 (10.1–12.3)	10.7 (9.4–12.1)	0.504
Serum triglycerides, mmol/L	0.92 (0.86–0.98)	0.98 (0.90–1.07)	0.83 (0.75–0.91)	0.010
Serum HDL-C, mmol/L	1.32 (1.28–1.36)	1.30 (1.25–1.35)	1.36 (1.29–1.42)	0.153
Serum apoB, g/L	0.71 (0.69–0.74); n = 189	0.73 (0.69–0.76); n = 108	0.70 (0.66–0.75); n = 81	0.228
24-h systolic BP, mm Hg	117 (116–118); n = 179	116 (114–117); n = 100	118 (116–119); n = 79	0.375[b]
24-h diastolic BP, mm Hg	69 (68–69); n = 179	68 (67–69); n = 100	69 (68–70); n = 79	0.326[b]

Data given as mean (95% CI) or, for the variables with skew distributions, as mean, median and range.
Abbreviation: BP, blood pressure.
[a]Mann-Whitney U-test for the differences between the girls and boys.
[b]Analysis of covariance adjusted for BMIadj and height (SD score).

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Table 3. Comparison Among IL-1Ra Tertiles

Variable	Tertile			*P* Value[a]
	Lowest (<195.0 pg/mL; n = 64)	Middle (195.1–286.0 pg/mL; n = 64)	Highest (>286.0 pg/mL; n = 63)	
Serum hs-CRP, mg/L				
Mean	0.19	0.33	1.45	<0.001
Median	0.09	0.09	0.70	<0.001[b]
Range	0.09–2.13	0.09–5.52	0.09-9.43; n = 59	
Serum insulin, mU/L	9.0 (8.0–10.0)	9.8 (8.9–10.6)	11.8 (10.5–13.0)	<0.001
Blood glucose, mmol/L	4.3 (4.2–4.4)	4.3 (4.3–4.4)	4.4 (4.3–4.4)	0.969
QUICKI	0.358 (0.352–0.364)	0.352 (0.346–0.357)	0.343 (0.337–0.349)	0.001 0.046[b]
Serum IGFBP-1, μg/L	74.7 (65.1–84.2)	67.0 (59.9–74.1)	56.6 (46.6–66.5)	0.001 0.030[b]
Serum SHBG, nmol/L	85.2 (77.0–93.4)	79.0 (71.7–86.3)	56.3 (49.0–63.7)	<0.001 <0.001[b]
Serum leptin, μg/L				
Mean	9.4	10.7	25.3	<0.001
Median	4.4	8.3	23.0	<0.001[b]
Range	0.3–52.4	0.9–41.0	0.8–101.5	
Serum adiponectin, mg/L	10.9 (9.4–12.3)	11.7 (9.9–13.4)	10.4 (9.1–11.7)	0.700
Serum triglycerides, mmol/L	0.81 (0.73–0.88)	0.95 (0.84–1.07)	0.99 (0.88–1.11)	0.037
Serum HDL cholesterol, mmol/L	1.40 (1.33–1.47)	1.31 (1.24–1.38)	1.26 (1.20–1.32)	0.016
Serum apoB, g/L	0.69 (0.64–0.74); n = 63	0.74 (0.69–0.78)	0.72 (0.67–0.76); n = 62	0.170
BMIadj, kg/m^2	19.5 (18.9–20.1)	20.5 (19.8–21.3)	24.3 (23.3–25.4)	<0.001 <0.001[b]
WHtR	0.41 (0.40–0.41)	0.42 (0.41–0.43)	0.47 (0.45–0.49)	<0.001 <0.001[b]
24h-systolic BP, mm Hg	116 (114–118); n = 60	117 (114–119); n = 61	118 (116–120); n = 58	0.449[c]
24-h diastolic BP, mm Hg	68 (66–69); n = 60	69 (68–70); n = 61	69 (68–70); n = 58	0.244[c]
Sex, F/M, no.	36/28	42/22	31/32	0.172[d]
Pubertal development,[e] early or late stage, no.	39/25	31/33	36/27	0.345[d]
Tanner B/G stage				0.652[d]
1	11 (4/7)	12 (7/5)	17 (3/14)	
2	28 (10/18)	19 (9/10)	19 (5/14)	
3	14 (12/2)	21(15/6)	15 (11/4)	
4	9 (8/1)	9 (8/1)	9 (9/0)	
5	2 (2/0)	3 (3/0)	3 (3/0)	

Data given as mean (95% CI); medians and ranges are given for the variables with skew distributions.
Abbreviation: B/G, breast/genital development.
[a]Independent-samples Kruskal-Wallis test, Bonferroni-adjusted *P* value, comparison between the lowest and the highest IL-1Ra tertile.
[b]Independent-samples Kruskal-Wallis test for the differences, Bonferroni-adjusted *P* value, comparison between the middle and the highest IL-1Ra tertile.
[c]Analysis of covariance adjusted for BMIadj and height (SDS).
[d]χ^2 test.
[e]Early stage of puberty: breast or genital scores 1–2; late stage of puberty: breast or genital scores 3–5.

significantly between these groups (0.01, 0.30, and 0.49 in the lowest, middle, and highest IL1-Ra tertiles, respectively, $P = 0.060$ by the Kruskall-Wallis test). Furthermore, the lowest serum HDL-C and the highest insulin and triglyceride concentrations were found in the highest IL1-Ra tertile and were significantly different from those in the lowest IL1-Ra tertile (Table 3). The concentrations of blood glucose, serum apoB, or adiponectin were not significantly different among the IL1-Ra tertiles. Sex distribution and pubertal development did not differ significantly among the IL-1Ra tertiles (Table 3). In sex-specific tertile analyses, the differences remained similar in the girls but were attenuated in the boys. However, the

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associations maintained the same trend in the boys as detected in the girls and in the whole study population (data not shown). In a binary logistic regression analysis adjusted for sex, pubertal development (early or late stage), birth weight SD score, and maternal PRE pregnancy history, higher leptin and hs-CRP concentrations, and WHtR associated independently with high IL-1Ra concentration. A 1-μg/L increase in leptin concentration, a 0.1-mg/L increase in hs-CRP concentration, and a 0.01 unit increase in WHtR were associated with a 1.08-, 1.30-, and 1.20-fold risk of a high IL-1Ra value, respectively (Table 4).

D. Factors Associated With Higher Serum hs-CRP Concentrations

The children in the higher hs-CRP group (*i.e.*, hs-CRP concentration >0.09 mg/L; n = 87) had higher BMIadj (22.6 vs 20.4 kg/m^2; $P = 0.002$), WHtR (0.45 vs 0.41; $P < 0.001$), and higher serum IL-1Ra (363.7 vs 207.9 pg/mL; $P < 0.001$), leptin (19.7 vs 11.2 μg/L; $P = 0.011$), and apoB (0.75 vs 0.68 g/L; $P = 0.018$) concentrations, and lower serum SHBG concentrations (66.0 vs 81.5 nmol/L; $P < 0.001$) than the children with lower hs-CRP (hs-CRP concentration ≤0.09 mg/L; n = 100). Pubertal development or sex distribution did not differ significantly between these groups.

In BMIadj-adjusted analyses, serum hs-CRP concentration correlated positively with serum IL-1Ra concentration in the whole study population ($r = 0.306$; $P < 0.001$; n = 184) and when the sexes were analyzed separately (girls: $r = 0.250$, $P = 0.009$, n = 105; boys: $r = 0.401$, $P < 0.001$, n = 79). A similar correlation between hs-CRP and IL-1Ra levels was found in WHtR-adjusted analyses in the whole study population ($r = 0.280$; $P < 0.001$; n = 184) and in the boys ($r = 0.403$; $P < 0.001$; n = 79), whereas the association in the girls was attenuated ($r = 0.140$; $P = 0.151$; n = 105). In a binary logistic regression analysis (adjusted for sex, early or late pubertal development stage, birth weight SD score, WHtR, and maternal PRE pregnancy history), higher IL-1Ra (OR, 1.09; 95% CI, 1.05 to 1.14; $P < 0.001$) and apoB (OR, 1.31; 95% CI, 1.08 to 1.60; $P = 0.006$) levels were associated independently with high serum levels of hs-CRP. The other covariates (*i.e.*, serum HDL-C, leptin, and SHBG) were not independently associated with high hs-CRP level in this analysis (data not shown). This model explained 37.8% of the variation in the dependent variable (*i.e.*, high hs-CRP level).

E. IL-1Ra and hs-CRP as Markers of Reduced Insulin Sensitivity and Unfavorable Lipid Profile

An ROC curve analysis was performed to compare IL-1Ra and hs-CRP levels as markers by which to identify the children with the lowest QUICKI values. The cut point of IL-1Ra level ≥226.6 pg/mL had a sensitivity of 72% and specificity of 64% [area under the ROC curve (AUC) for IL-1Ra, 0.676; 95% CI, 0.595 to 0.757; $P < 0.001$] to detect the children with the lowest QUICKI values. BMIadj rose slightly above IL-1Ra in ability to discriminate the children with the lowest IS (AUC for BMIadj, 0.691; 95% CI, 0.614 to 0.768; $P < 0.001$), whereas IL-1Ra was a more reliable marker than WHtR (AUC for WHtR, 0.626; 95% CI,

Table 4. Factors Associating With High IL-1Ra (Highest IL-1Ra Tertile, n = 56) in the Whole Study Population

Covariate	Regression Coefficient	Significance	OR	95% CI for OR
High serum hs-CRP (0.1 mg/L)	0.259	<0.001	1.30	1.13–1.49
High serum leptin, μg/L	0.081	0.003	1.08	1.03–1.14
High WHtR (0.01 units)	0.183	0.030	1.20	1.02–1.42
High blood glucose (0.1 mmol/L)	0.076	0.423	1.08	0.90–1.30
High serum triglyceride levels (0.1 mmol/L)	0.106	0.095	1.11	0.98–1.26

Highest IL-1Ra tertile, n = 56. Results of the binary logistic regression analysis (n = 181) adjusted for sex, pubertal stage (Tanner stages of breast/genital development 1–2/3–5), birth weight (SD score) and maternal PRE pregnancy history; variation explained by the model 68.6%.

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0.540 to 0.713; $P = 0.004$). The cut point of hs-CRP level ≥0.69 mg/L had a sensitivity of 31% and a specificity of 85%, with no statistical significance (AUC for hs-CRP, 0.541; 95% CI, 0.450 to 0.632; $P = 0.361$). Levels of IL-1Ra and hs-CRP failed to be useful in detecting the children with the highest triglyceride and lowest HDL-C concentrations (data not shown).

Finally, independent contributions of IL-1Ra, hs-CRP, and BMIadj to the variance in IS and lipid variables were evaluated in multivariable regression models (adjusted for sex and pubertal developmental stage). In these analyses, only BMIadj could significantly predict low IS (measured by QUICKI) or HDL-C level, whereas serum IL-1Ra and hs-CRP levels had no independent association with them (Table 5). The positive association between BMIadj and triglycerides was significant when hs-CRP was entered in the model (Table 5).

3. Discussion

The results of this study revealed that the children with the highest serum IL-1Ra concentrations had lower IS, higher serum leptin and hs-CRP levels, and a less favorable lipid profile than the children with lower serum IL-1Ra values. In a logistic regression analysis, high leptin, hs-CRP, and WHtR values associated independently with high IL-1Ra level. The ROC curve analysis showed that IL-1Ra level had a weak, but statistically significant ability to predict low IS estimated by QUICKI. However, in a multivariable regression model, IL-1Ra level had no independent association with IS.

Insulin resistance is associated with altered levels of cytokines, especially in visceral adipose tissue [1, 3]. In obesity, adipose tissue macrophages release IL-1β, which inhibits adipogenesis, impairs insulin signaling in adipocytes, and increases insulin resistance [4]. The secreted form of IL-1Ra, produced particularly by monocytes, macrophages, dendritic cells, neutrophils, and epithelial cells in the adipose tissue [24], is a natural counterregulator of IL-1β [3, 6, 7]. Its circulating concentrations are >100-fold higher compared with those of IL-1β [6]. Elevated IL-1Ra concentrations have been observed up to 13 years before the onset of type 2 diabetes [4] and are associated with 10-year diabetes risk [25], but they diminish in patients with longstanding type 2 diabetes [26]. In the study of Grossmann *et al.* [10] including >15,000 adults, IL-1Ra concentrations gradually increased from patients with normoglycemia to those with prediabetes and further in those with type 2 diabetes.

Several studies have reported a positive correlation between IL-1Ra concentration and BMI, lean body mass, waist circumference, insulin, and insulin resistance indexes [9, 12, 27].

Table 5. Multivariable Regression Analysis of Serum Inflammatory Markers, Adiposity Variables, and Insulin Sensitivity Markers

Model/Dependent Variable	Independent Variable	r^{2a}	β	P Value
Model 1, n = 191				
QUICKI	IL-1Ra	0.270	−0.015	0.843
	BMIadj		−0.456	<0.001
Triglyceride levels	IL-1Ra	0.104	0.145	0.076
	BMIadj		0.154	0.070
HDL-C level	IL-1Ra	0.140	−0.011	0.893
	BMIadj		−0.256	0.002
Model 2, n = 187				
QUICKI	hs-CRP	0.272	−0.016	0.811
	BMIadj		−0.461	<0.001
Triglyceride levels	hs-CRP	0.090	0.085	0.259
	BMIadj		0.187	0.018
HDL-C level	hs-CRP	0.151	−0.122	0.095
	BMIadj		−0.211	0.006

Sex and pubertal developmental stage (breast/genital development 1–5) were entered as covariates. Skewed variables were logarithmically or square root–transformed before testing.

[a]The variance explained by the model.

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Our study findings are in line with results of these studies: The children with the highest IL-1Ra concentrations had higher BMIadj and WHtR than those with lower IL-1Ra concentrations, and a similar sex distribution and pubertal developmental stage. Furthermore, in a binary logistic regression analysis, higher WHtR was associated with high IL-1Ra concentration. The children in the highest IL-1Ra tertile had also higher serum insulin and lower QUICKI, serum IGFBP-1 and SHBG values. Circulating IGFBP-1 and SHBG levels have been associated with IS in healthy adults [28] and SHBG concentrations with IS and metabolic risk during puberty [29].

The acute-phase protein CRP is a sensitive marker of the body's inflammatory burden and a downstream surrogate biomarker for IL-1β activity. Its high-sensitivity assays are accurate enough to detect low-grade inflammation [5]. Thus, hs-CRP is used in CVD risk prediction in adults [5] and youth [30]. In the current study, serum hs-CRP levels were higher in children in the highest IL-1Ra tertile compared with those in the lower tertiles. In addition, a positive association between IL-1Ra and hs-CRP levels was found independently of BMIadj and WHtR. Recently, Bugge *et al.* [30] did not find any correlation between IL-1Ra and CRP levels in Danish adolescents, whereas a positive correlation has been demonstrated in prepubertal children [31] and in adults [32]. CRP levels associate with central adiposity and BMI [30, 33, 34], and elevated CRP concentrations have been reported in obese children and adolescents as a marker of subclinical inflammation [33, 34]. In the current study, the children with higher hs-CRP values had higher BMIadj and WHtR. They also had higher serum IL-1Ra, leptin, and apoB, and lower SHBG concentrations. A negative association between CRP and SHBG independently of BMI has been described in children and adolescents [35].

Lipids regulate metabolism and inflammation, and hyperlipidemia induces, in part, peripheral tissue insulin resistance and contributes to the development of atherosclerosis [2]. In this study, the children in the highest IL-1Ra tertile had lower HDL-C and higher triglyceride concentrations compared with those in the lowest tertile. In line with this, circulating IL-1Ra levels were independently associated with low HDL-C and high triglyceride concentrations in healthy, nondiabetic men [27]. ApoB is considered to more accurately predict CVD risk than total cholesterol and low-density lipoprotein cholesterol levels, because it indicates the total number of atherogenic particles in circulation [36]. A positive association between levels of apoB and plasma IL-1Ra [37] or CRP [38] has been described in overweight or obese adults, and elevated apoB levels have been reported in subjects with insulin resistance or type 2 diabetes [39]. In this study of healthy 12-year-old children, we were not able to detect any difference in serum apoB concentrations among the IL-1Ra tertiles. Instead, higher apoB concentrations were found in children with higher hs-CRP levels than in those with lower hs-CRP levels, and higher apoB level predicted higher hs-CRP level in a logistic regression analysis.

We used ROC curve analyses to compare IL-1Ra and hs-CRP levels as markers for identifying the children with the lowest QUICKI values. IL-1Ra level had a relatively low, but statistically significant discrimination value in detecting low IS, whereas hs-CRP level did not distinguish the children with the lowest QUICKI value. These findings suggest IL-1Ra may be a more sensitive serum marker than hs-CRP in detecting reduced IS in 12-year-old children. However, the associations between IS and levels of IL-1Ra or hs-CRP were strongly weight related: The regression analyses revealed that neither IL-1Ra nor hs-CRP levels had an independent contribution to reduced IS. Furthermore, both IL-1Ra and hs-CRP levels failed to predict low HDL-C and high triglyceride concentrations.

The satiety hormone leptin controls energy homeostasis and also regulates immune response [2]. Its serum concentrations are associated with body-fat storage and circulating inflammatory markers [1]. In the current study, the highest serum leptin concentrations (with highest BMIadj and WHtR) were found in the highest IL-1Ra tertile and in the higher hs-CRP group. In logistic regression analyses, both high leptin and high WHtR values were associated independently with high IL-1Ra concentration, whereas they were not associated with high hs-CRP concentration, which indicates IL-1Ra level may be a more sensitive serum marker than hs-CRP level for obesity-related low-grade inflammation.

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In this study, we did not find a difference in serum IL-1Ra or hs-CRP concentrations between girls and boys. This is in accordance with Cullup *et al.* [40], who reported that sex, age, and menstruation status have no effect on IL-1Ra levels in healthy adults. Furthermore, similar to our findings, Andersen *et al.* [41] did not find sex differences in CRP concentrations among children 9 to 10 years old, whereas Cook *et al.* [33] reported that 10- to 11-year-old girls had higher hs-CRP values compared with boys (without pubertal stage adjustment).

Low birth weight and maternal preeclampsia predispose to later cardiovascular morbidity [13–15]. In the present cohort, we did not find any significant difference in serum IL-1Ra and hs-CRP concentrations or other measured metabolic parameters in children in the SGA, AGA, or PRE pregnancy groups. This finding suggests that other factors than small birth size or maternal preeclampsia are more important in influencing the degree of low-grade inflammation in 12-year-old children.

The strengths of this study include detailed anthropometric data of the study subjects at birth and at the age of 12 years, and numerous biochemical measurements. However, there are also some weaknesses. The study age of 12 years is challenging because of the variable timing in the pubertal development. We tried several ways to exclude the possible influence of this on our results. We verified that the pubertal developmental stage did not differ significantly among the compared subgroups (*i.e.*, the IL-1Ra tertiles and the hs-CRP groups) and adjusted the logistic regression analyses for the pubertal developmental stage. Despite controlling several confounding factors, the one-time measurement of IS could be a potential limitation in our study. Fasting insulin level alone or in combination with fasting glucose level is not an optimal measure for assessing individual IS, but fasting insulin and glucose values may be applicable in studies with well-defined cohorts [42]. We used QUICKI as a surrogate marker of IS. QUICKI correlates quite well with the glucose clamp method [43]. Unfortunately, we did not have data on other factors influencing IS, such as dietary habits and the frequency and intensity of exercise.

4. Conclusion

The children with the highest IL-1Ra concentrations had lower IS; higher serum hs-CRP, BMIadj, and WHtR values; and a less favorable lipid profile than those with the lowest IL-1Ra values. These findings suggest high IL-1Ra concentrations are associated with unfavorable metabolic features in 12-year-old children. IL-1Ra concentration was a better marker of low IS in the children than was hs-CRP concentration, but the real value of IL-1Ra measurements in predicting CVD risk in children remains to be determined.

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