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Increased body fat and reduced insulin sensitivity are associated with impaired endothelial function and subendocardial viability in healthy, non-Hispanic white adolescents

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Abstract

Background: Cardiovascular disease has its origins in adolescents. Endothelial dysfunction, arterial stiffness, and decreased endocardial oxygen supply: demand ratios are early functional markers of cardiovascular risk. The goal of this study was to determine the relationships of these markers to physical, inflammatory, and metabolic markers in healthy non-Hispanic, white adolescents.

Methods: Thirty-four of the 75 subjects were female. Mean age was 15.0 ± 1.7 years and mean body mass index (BMI) was 22.0 ± 5.8 kg/m^2 (mean ± SD). Reactive hyperemia was measured using venous occlusion plethysmography. Arterial tonometry was used to measure the augmentation index (AIx_{75}) and the Buckberg subendocardial viability ratio. Blood samples were taken to measure inflammatory and lipid markers and oral glucose tolerance test was used to assess insulin sensitivity.

Results: Reactive hyperemia decreased as body mass and fat mass increased. It also decreased with increasing neutrophil count. The Buckberg index was higher in males and was positively related to insulin sensitivity even when accounting for age, sex, and resting heart rate. AIx_{75} was not related to any of the other variables.

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AUTHOR CONTRIBUTIONS

R.P.H. designed and oversaw performance of the study, performed statistical analysis, wrote and approved the final manuscript. M.M.C. helped perform the studies and reviewed the manuscript, D.Z. performed laboratory measurements and reviewed the manuscript, and C.Y.Y. helped design the study, oversaw laboratory measurements, and reviewed the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

Conclusions: These results demonstrate that increased fat mass and decreased insulin sensitivity are related to poorer vascular function and cardiac risk in adolescents before the development of actual cardiovascular disease.

Keywords

adolescents; arterial stiffness; inflammation; insulin sensitivity; reactive hyperemia

1 | INTRODUCTION

While cardiometabolic diseases such as coronary artery disease, atherosclerosis, hypertension, and type 2 diabetes, usually do not produce significant mortality and morbidity until adulthood, there is clear evidence that these diseases have their origins in childhood and adolescence. Studies of soldiers killed in Korea at average age of 22 years showed 77% had significant atherosclerosis.[1] Autopsy studies of 15–34 year old's who died from accidental or non-accidental trauma demonstrated raised coronary artery lesions in individuals as young as 15 years with significantly increasing prevalence over time.[2] With the rising incidence of obesity associated with poorer diet and less physical activity in children and adolescents, it is important that we study these diseases early in their course if we are to prevent future cardiometabolic morbidity and mortality.

In healthy adults insulin resistance has been closely linked to impaired cardiac function, increased arterial stiffness,[3,4] and decreased endothelial function.[5–7] In addition, hyperinsulinism has been shown to impair endothelial function.[8,9] In adolescents, where cardiovascular disease has its origins, less is known. Most articles report decreased endothelial function and/or increased arterial stiffness in obese adolescents[10–15] although there may be potential racial differences.[16,17] One study reported increased flow mediated dilation with increased adiposity in this age group.[18] These studies have found variable relationships to insulin sensitivity and other measures of cardiovascular risk. Diastolic dysfunction in obese adolescents has been related to increased insulin resistance.[19] We, first, hypothesized that adiposity and insulin resistance would be associated with impaired vascular and cardiac functional markers in adolescents. Second, as adolescent females have been shown to have lower insulin sensitivity than males of similar body mass index (BMI) and Tanner stage,[20] we hypothesized that they should, also, have reduced cardiac and vascular function compared to adolescent males. Thus, the goal of this study was to investigate the relationships of vascular and cardiac functional risk markers to physical, inflammatory, and metabolic risk markers in healthy adolescents.

2 | METHODS

2.1 | Subjects

Seventy-five, white, non-Hispanic identifying adolescents between the ages of 12 to 17 years 11 months were recruited to participate. Subjects were medication free for at least 2 weeks except for oral contraceptives in females. Subjects with a history of autoimmune endocrine, connective tissue disease, hematologic disease, renal disease, or malignancy were excluded. The protocol was approved by the Nationwide Children's Hospital Institutional Review

Board and written informed assent from the subject and informed consent from a parent or guardian were obtained.

2.2 | Protocol

The protocol was registered on ClinicalTrials.gov, NCT02821104.

2.2.1 | Study visit—Subjects arrived at the Clinical Research Center of the Wexner Medical Center at The Ohio State University at 08:00 after overnight fast beginning at 22:00 the night before the study. Upon arrival, a brief history was taken and physical examination was performed.

2.2.2 | Anthropometrics—Height (Stadiometer: 216 Accu-Hite Measuring Device, Seca North America, Chino, California), weight (SECA 1360 Wireless scale) were measured. Waist circumference was measured to the nearest 0.1 cm at the narrowest place between the lowest rib and the iliac crest at the end of normal exhalation with a spring-loaded, inelastic measuring tape.[21] Body fat percentage was measured using air displacement plethysmography in the BODPOD (COSMED USA Inc., Concord, California). Fat mass index (FMI) was calculated as total body fat weight/height^2 and fat free mass index (FFMI) as total non-fat weight/height.^{2}

2.2.3 | Arterial tonometry—Arterial tonometry (SphygmoCor, AtCor Medical, Inc., Itasca, Illinois) was used to determine the augmentation index, as a measure of vascular stiffness. Results were corrected to heart rate of 75 (AIx_{75}). The software, also, calculates the Buckberg subendocardial viability ratio, which is a measure of cardiac oxygen supply: demand ratio.[3,22]

2.2.4 | Endothelial function—Endothelial function was measured using postocclusion, shear stressinduced, endothelially mediated vasodilation (reactive hyperemia) after a 20-minute rest period. Strain gauge venous occlusion plethysmography (Hokanson AI6 plethysmograph, DE Hokanson, Inc., Bellevue, Washington) was used to measure forearm blood flow (FBF) and forearm vascular resistance (FVR) before and after upper arm occlusion. This method of testing endothelial function assesses resistance vessel function.[17]

The reactive hyperemia response was quantified as percent decrease in FVR using the following protocol. Two minutes of baseline FBF were measured after which the upper arm cuff was inflated to 200 mmHg pressure for 5 minutes to occlude arterial inflow. It was then released and FBF was measured for 1 minute. FVR were calculated by dividing mean arterial blood pressure by FBF. Arterial blood pressure was measured using automated sphygmomanometer. Mean intra-observer coefficient of variation (CV) for FBF before upper arm occlusion is 5.1% and 7.4% after upper arm occlusion.

2.2.5 | Blood sampling—After completion of the test of endothelial function, an intravenous catheter was placed in one arm for initial blood sampling and for oral glucose tolerance testing. Twenty-five milliliters of blood was taken from each study subject for measurement of fasting plasma glucose, insulin, lipid, interleukin-6 (IL6) and endothelin 1 concentrations, and high sensitivity c-reactive protein (CRP), and plasminogen activator

inhibitor-1 (PAI1) levels. White blood cell and neutrophil counts and IL6 concentrations and CRP levels are markers of inflammation, which are clearly increased in individuals at increased cardiometabolic risk.[23,24] PAI1 was used to assess clotting risk. Endothelin 1 is an endothelially-produced vasoconstrictor and has been shown to be a biochemical marker for endothelial dysfunction.[25]

2.2.6 | Insulin sensitivity and secretion—Subjects were given 1.75 g/kg of oral glucose (up to a maximum of 75 g). Blood samples for measurement of plasma glucose and insulin concentrations were drawn every 30 minutes for 120 minutes. Insulin sensitivity was assessed using the Matsuda Index (MAT = 10 000/√[fasting glucose × fasting insulin] × [mean glucose × mean insulin]) while insulin secretion was assessed as the change in insulin divided by the change in glucose from 0 to 30 minutes or insulinogenic indexe(IGI = ΔI_{0-30} /ΔG_{0-30}).[26] Disposition index (DI) was calculated by multiplying both these values (DI = IS × IGI).[26] Disposition index is an important predictor of future type 2 diabetes.[27]

2.2.7 | Cardiovascular measures, glucose, and insulin—Cell differentials (neutrophils, total white blood cells) from EDTA-peripheral blood were measured by a Sysmex Kx-21 N cell counter (Sysmex, Lincolnshire, Illinois). Plasma lipids, glucose, insulin, IL6, CRP, endothelin 1, fibrinogen, plasminogen activator inhibitor antigen (PAI1), were measured in the Clinical Research Center CORE laboratory.

2.2.8 | Statistical analysis—All analysis was done using non-parametric testing. Mann Whitney *U* test was used for comparison between sexes. The relationships of cardiovascular function measures (reactive hyperemia, Aix_{75}, Buckberg index) to measures of body habitus and physical (heart rate, blood pressure), biochemical (lipids, inflammatory markers, endothethelin-1, PAI-1) and carbohydrate metabolism (fasting insulin, insulin sensitivity, insulin secretion, disposition index) cardiometabolic risk factors were assessed using robust, rank order regression analysis with age and sex included in all models. When the cardiovascular function measures were significantly related to both body habitus and other cardiometabolic risk factors additional models were constructed to include both measures in the model. The cut-off point for outliers was 3 and 100 iterations were performed. Results are presented as 95% confidence intervals (CI). All analysis was performed using Systat 13 (Systat, Inc., Evanston, Illinois).

3 | RESULTS

3.1 | Demographics

Thirty-four of the 75 subjects were female. Mean age was 15.0 ± 1.7 years and mean BMI was 22.0 ± 5.8 kg/m^2 (mean ± SD). Mean BMI percentile was 53.8 ± 28.7. Sixty-two subjects were lean (BMI percentile<85) and nine were considered obese (BMI percentile≥95). Table 1 shows values for male and female subjects. Males had increased waist circumference and FFMI compared to females and females had increased percent body fat and FMI.

3.2 | Cardiovascular measures

Table 2 shows the cardiovascular measures in each sex. Females, for the most part, had increased cardiometabolic risk compared to males as indicated by lower Buckberg and Matsuda indices, higher neutrophil counts, and PAI-1 levels although they, also, had higher HDL concentrations and disposition index.

3.3 | Cardiovascular measures and body habitus

The reactive hyperemic response was not related to age or sex and worsened as BMI, BMI percentile, percent body fat, waist circumference and FMI increased (Figure 1). It was not related to FFMI. AIx_{75} was not related to age, sex or any measure of body habitus. The Buckberg index decreased with increasing percent body fat ($\beta = -1.03$; 95% CI: −2.04 to −0.02). Interestingly, including percent body fat in the regression analysis eliminated the sex difference in MAT.

3.4 | Cardiovascular measures and risk factors

Reactive hyperemia worsened with increased neutrophil count and fasting insulin concentration (Table 3: Models 1 and 2) but was not related to white blood cell count, CRP, IL6, lipids, MAT, IGI, DI, endothelin 1, or PAI-1. When percent body fat was included in the equation with either neutrophil count or fasting insulin concentration all relationships approached significance (Table 3: Models 3 and 4). If percent body fat, neutrophil count and fasting insulin were included, the relationship to neutrophil count was significant and the other relationships approached significance (Model 5). AIx_{75} was not related to any of the measures.

The Buckberg index was negatively related to WBC ($\beta = -4.80$; 95% CI: −9.53–0.07) and was positively related to MAT (Figure 2) but was not related to WBC, neutrophil, count, CRP, IL-6, fasting insulin, IGI, endothelin 1 or PAI-1. Sex remained a significant predictor of the Buckberg index when either percent body fat or MAT was individually included in the regression analysis (Table 3, Models 6 and 7). As both MAT and the Buckberg index were significantly related to percent body fat modeling was, also, done with both included as independent variables (Model 9). Interestingly, the relationship to MAT remained significant while the relationship to percent fat was no longer significant. Sex remained significant.

3.5 | Cardiovascular risk factors and pulse and blood pressure

Reactive hyperemia and AIx_{75} were not significantly related to heart rate or blood pressure. The Buckberg index was negatively related to heart rate (Model 8) and inclusion of heart rate in the model eliminated the sex effect. When heart rate was included in the model for the Buckberg index both heart rate and MAT were significant predictors but sex was not (Model 10). Buckberg was not related to blood pressure.

4 | DISCUSSION

If cardiovascular disease is to be prevented, it is important that we understand the early pathophysiology of the process in adolescents before overt disease and its complications may confound potentially mechanistic relationships. The current study adds to our

understanding regarding the relationships of vascular and cardiac perfusion risk factors to traditional and non-traditional metabolic risk factors.

Endothelial dysfunction is thought to be one of the earliest predictors of future atherosclerotic disease, as the endothelium plays an important role in all phases of atherosclerosis, ranging from affecting tissue blood supply to clotting and inflammation.[28] Our results demonstrate that the primary association of endothelial function in this age group is with obesity as we found consistent decreases in the reactive hyperemic response with increasing body size. Increasing fat mass appears to be the primary culprit for this relationship as the reactive hyperemic response was closely related to FMI, but not FFMI. In adults, the presence of insulin resistance in obesity may mediate this relationship.[5–7] The relationship of endothelial function to insulin sensitivity in adolescents is less clear as a relationship has been found in some,[29,30] but not all studies,[17] and may be dependent on how endothelial function and insulin sensitivity are assessed.[31] In the current study we did not find a relationship between endothelial function and insulin sensitivity. The Matsuda index has been found to closely correlate with results from hyperinsulinemic clamp in adolescents.[32] Our small number of extremely obese, insulin resistant subjects may minimize the relationship between insulin sensitivity and endothelial function.

Interestingly, subjects with higher fasting insulin levels had poorer reactive hyperemic responses in spite of the lack of relationship to either MAT or IGI from the OGTT. This relationship continued to approach significance even when adiposity was included in the model. Potential reasons for this include the fact that fasting insulin is more closely related to hepatic than peripheral or total body insulin sensitivity[33] and that chronic high fasting insulin may have a more deleterious effect on endothelial function than increased postload responses. In addition, we only studied non-Hispanic white subjects. We, previously, found a significant relationship between insulin secretion in response to intravenous glucose and endothelial function in black, but not white, subjects.[17]

The decreasing reactive hyperemic response with increasing neutrophil count in adolescents is an important new finding. This relationship continued to approach significance even when percent body fat was included in the model, indicating the relationship is not solely due to obesity. Increased white cell counts have been shown to predict arterial stiffness in adults in a variety of conditions[34] and increased neutrophil counts predict future cardiovascular disease in adults.[35] The increased neutrophil counts are thought to be evidence of increased endothelial inflammatory damage. The relationship between reactive hyperemia and neutrophils in the current study indicates that increased neutrophil count in adolescents is an indicator of increased cardiovascular risk although we did not find a relationship to AIx_{75}. We did not find relationships between reactive hyperemia or AIx_{75} and either of the two inflammatory biochemical markers measured, IL-6 and CRP. This may indicate that we used the wrong markers. Tomosa et al.,[30] however, found no relationship between reactive hyperemia and tissue necrosing factor-α, but did find a relationship for AIx_{75}. It is, also, possible that these relationships may not develop until a later age. Both of the measured markers in our study are significantly related to arterial stiffness markers in adults.[34] Kapiotis et al.[10] found higher CRP and IL-6 in obese and morbidly obese children. It is,

again, possible these relationships might have been found had we included a larger number of obese subjects.

Another important new finding is the significant relationship between the Buckberg subendocardial viability ratio and insulin sensitivity, as measured using OGTT and the Matsuda Index. The Buckberg index assesses the ratio of oxygen supply to oxygen demand by the myocardium[22] with increasing ratios indicating reduced cardiac risk. Koshdel et al.[3] found reduced Buckberg indices in insulin resistant adult men with the metabolic syndrome, but did not report a direct relationship between the Buckberg index and insulin sensitivity. Franssen et al.[19] reported a direct relationship of insulin resistance and/or hyperinsulinemia to several echocardiographic parameters of diastolic dysfunction in a mixed group of obese and lean adolescence and also a relationship to delayed heart rate recovery following exercise.

Importantly, the relationship between the Buckberg index and insulin sensitivity remained significant even when percent body fat was included in the equation. In fact, the relationship to percent body fat was no longer significant in this model. The reasons for the relationship between the Buckberg index and insulin sensitivity are not immediately clear. One possibility might be both being indicators of increased physical fitness which we did not directly measure. The Buckberg index was closely and negatively related to heart rate which might support this possibility as reduced resting heart rate is associated with improved physical fitness.[36] Peterson et al.[37] found a significant relationship between physical inactivity and increased cardiometabolic risk in adolescents. However, we found that both insulin sensitivity and resting heart rate significantly predicted the Buckberg index when both were included as independent variables. This suggests that the association between insulin sensitivity and the Buckberg index are, at least partially, independent of physical fitness.

Previous studies have found varied sex differences in cardiovascular risk factors in adolescents consistent with those found in this study.[17,37–39] Peterson et al.[37] compiled an overall metabolic risk score, however, and found no sex difference in spite of differences in individual factors. This is consistent with our findings of differences suggestive of both increased and decreased risk in both sexes. Our study adds to this by demonstrating lower cardiac oxygen supply: demand ratio in females. Differences in physical fitness may be partially responsible as the sex difference was no longer significant when heart rate was included in the model. This is unlikely to be the only cause, however, as the 95% confidence intervals were still strongly positive for a sex effect. When both heart rate and insulin sensitivity were included in the model the sex effect was clearly lost. This may be due to loss of statistical power, although as mentioned the relationships to heart rate and insulin sensitivity remained significant. The fact that some females were using oral contraceptives may confound our findings regarding insulin sensitivity although previous studies have demonstrated lower insulin sensitivity in adolescent females without the use oral contraceptives.[20] No sex differences in vascular function were found.

Cross-sectional relationships do not demonstrate causality and our study was limited to non-Hispanic white adolescents, so the results may not be applicable to other racial groups.

Importantly, our results do demonstrate the adverse effects of increased fat mass, inflammation, and insulin resistance on functional cardiovascular risk, which are present even in adolescents before the development of cardiovascular disease.

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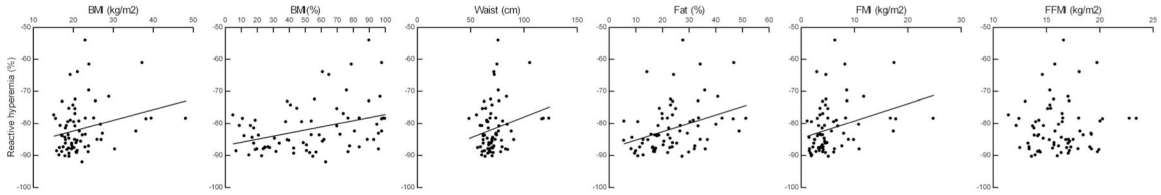

FIGURE 1.

Relationships between reactive hyperemia and measures of body habitus in healthy, non-Hispanic white adolescents. BMI: β = 0.32, 95%CI: 0.05–0.58; BMI (%): β = 0.071, 95%CI: 0.027–0.12; waist circumference β = 0.14, 95% CI: 0.02–0.27; Fat (%) β = 0.22, 95% CI: 0.05–0.40; FMI: β = 0.45, 95% CI: 0.07–0.84; FFMI: β = 0.54, 95% CI = −0.27–1.34. BMI, body mass index

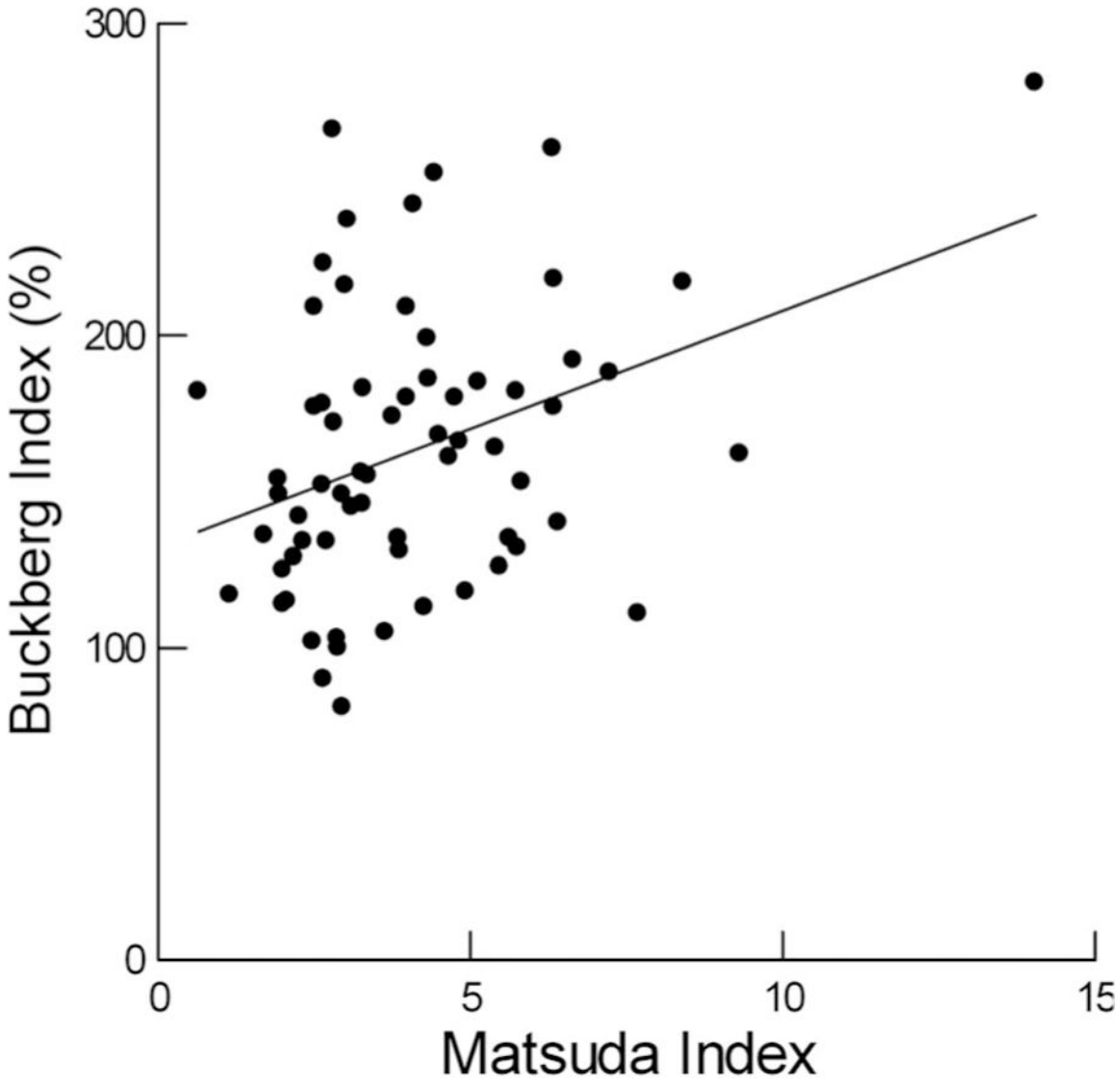

FIGURE 2.

Relationship of Buckberg index to insulin sensitivity in healthy, non-Hispanic white adolescents. β = 5.89, 95% CI: 1.22–10.57

TABLE 1

Demographic and body habitus values by sex (mean ± SD)

	Girls (n = 34)	Boys (n = 41)
Age (years)	15.4 ± 1.6	15.0 ± 1.7
BMI (kg/m^2)	22.3 ± 5.8	21.7 ± 5.9
BMI percentile	53.9 ± 30.6	53.2 ± 27.4
Waist circumference (cm)	70.2 ± 13.1 *	74.6 ± 13.0 *
Body fat (%)	28.4 ± 8.2 *	20.2 ± 11.0 *
FMI (kg/m^2)	6.70 ± 3.10 *	4.86 ± 4.61 *
BMI (kg/m^2)	15.5 ± 2.2 *	16.9 ± 2.5 *

Abbreviations: BMI, body mass index; FMI, fat mass index.

* $P < .05$ for sex difference.

TABLE 2

Cardiovascular measures according to sex (mean ± SE)

	Girls(n = 34)	Boys (n = 41)
Heart rate (beats/min)	69.7 ± 2.2	63.7 ± 2.1
SBP (mmHg)	113 ± 2	116 ± 2
DBP (mmHg)	65 ± 1	64 ± 1
Reactive hyperemia (%)	−80.8 ± 1.3	−82.5 ± 1.3
AIx_{75} (%)	−1.03 ± 3.71	−5.29 ± 3.32
Buckberg index (%)	145 ± 7*	175 ± 7*
White blood cell (cell/μl)	7.42 ± 0.30	7.02 ± 0.36
Neutrophil (cell/μl)	4.35 ± 0.27*	3.74 ± 0.26*
IL-6 (mg/ml)	0.572 ± 0.061	0.776 ± 0.132
CRP (mg/L)	2.40 ± 0.72	1.04 ± 0.37
HDL (mg/dl)	55 ± 3*	49 ± 2*
LDL (mg/dl)	80 ± 4	71 ± 3
Triglycerides (mg/dl)	81 ± 15	64 ± 5
Insulin sensitivity	3.67 ± 0.31*	4.78 ± 0.41*
Insulin secretion (μU dl/mg ml)	1.80 ± 0.19*	0.96 ± 0.10*
Disposition index	5.74 ± 0.59*	4.33 ± 0.56*
Endothelin 1 (pg/ml)	2.11 ± 0.09	2.02 ± 0.08
PAI-1 (ng/ml)	4.44 ± 0.54*	3.19 ± 0.35*

*$P < .05$ for sex difference.

TABLE 3

Beta coefficients for the relationship of Reactive Hyperemia and Buckberg subendocardial viability ratio to age, sex, and cardiovascular risk factors in healthy, non-Hispanic white adolescents

Reactive hyperemia					Buckberg index				
Model	**Intercept**	**Independent variable**	**β**	**95% confidence interval**	**Model**	**Intercept**	**Independent variable**	**β**	**95% confidence interval**
1	−81.7	Age	−0.43	−1.46–0.581	6	59.2	Age	5.53	−0.32–11.4
	…	Sex	−0.60	−4.06–2.86		…	Sex	23.2	2.44–43.9
	…	Neutrophils	1.41	0.33–2.49		…	%Body fat	−1.01	−2.00–0.024
2	−76.3	Age	−0.44	−1.45–0.58	7	4.46	Age	4.88	−1.25–11.0
	…	Sex	−1.70	−5.14–1.74		…	Sex	33.6	13.0–54.2
	…	Fasting insulin	0.17	0.008–0.33		…	Insulin sensitivity	5.89	1.22–10.6
3	−90.6	Age	−0.27	−1.36–0.82	8	244	Age	2.57	−1.24–6.38
	…	Sex	1.43	−2.80–5.66		…	Sex	11.6	−0.91–24.0
	…	% Body fat	0.11	−0.004–0.40		…	Heart rate	−2.25	−2.74–1.76
	…	Neutrophils	0.58	−0.012–2.32		…	…	…	…
4	−86.6	Age	−0.22	−1.29–0.85	9	9.67	Age	5.24	−1.16–11.6
	…	Sex	0.54	−3.66–4.75		…	Sex	28.3	4.34–52.3
	…	% Body Fat	0.18	−0.049–0.41		…	%Body fat	−0.087	−1.51–1.33
	…	Fasting insulin	0.15	−0.047–0.34		…	Insulin sensitivity	6.54	0.76–12.3
5	−91.1	Age	−0.26	−1.34–0.82	10	252	Age	2.26	−1.19–5.71
	…	Sex	1.33	−2.86–5.52		…	Sex	6.61	−4.31–17.5
	…	% Body fat	0.12	−0.074–0.39		…	Insulin sensitivity	3.49	1.03–5.95
	…	Neutrophils	1.17	0.018–2.33		…	Heart Rate	−2.36	−2.87–1.86
	…	Fasting insulin	0.16	−0.035–0.35		…	…	…	…