Relationship between Cardiovascular Risk Factors and Adipokines in Adolescents

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Key Words

Blood pressure · Cytokines · Lipids · Obesity · Adolescents

Abstract

This study evaluated the associations of adipokines with cardiovascular risk factors. **Subjects/Methods:** 60 normal weight (BMI $\leq$ 75th percentile) and 60 overweight (BMI $\geq$ 95th percentile) adolescents aged 10–14 years. Resting systolic and diastolic blood pressures (SBP, DBP) and waist circumference were obtained in duplicate. Circulating adiponectin, resistin, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) were measured from fasting plasma samples. **Results:** Simple correlations showed that SBP was significantly related ($p < 0.05$) to adiponectin ($r = -0.185$), resistin ($r = 0.207$), and IL-6 ($r = 0.238$); HDL was significantly related to adiponectin ($r = 0.398$) and TNF- α ($r = -0.227$). TG was only related to adiponectin ($r = -0.292$, $p < 0.05$). Multiple regression models controlling for puberty and ethnicity indicated that adiponectin ($R^2 = 0.152$, $p < 0.05$), resistin ($R^2 = 0.152$, $p < 0.05$), and IL-6 ($R^2 = 0.170$, $p < 0.05$) were associated with SBP. The association between adiponectin and HDL was stronger in normal weight versus overweight adolescents ($R^2 = 0.336$, $p < 0.05$). None of the other models showed differences in the associations by weight status. **Conclusions:** In adolescents, SBP but not DBP

was associated with most adipokines. HDL, but not TC, was also associated with some adipokines. TG were only associated with adiponectin. Associations were mostly related to adiposity.

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Introduction

In children and adults, the association between obesity and the presence of cardiovascular disease risk factors and insulin resistance is well established [1–4]. However, the mechanisms relating adiposity to the atherosclerotic process are still under study [5]. One of several proposed hypotheses is that adipose tissue acts as a secretory gland releasing hormones and adipokines with pro- or anti-inflammatory activity [5, 6]. These adipokines in turn affect either glucose or fat transport/utilization, triglycerides (TG) production, or endothelial function in a negative or positive way [5, 7–10].

Tumor necrosis factor- α (TNF- α), adiponectin, and resistin have been previously associated with insulin resistance and obesity in youth [11], as well as other cardiovascular disease risk factors such as blood pressure (BP) [12–14]. Elevated interleukin-6 (IL-6) concentrations have been associated with cardiovascular disease in adults [15] and higher IL-6 concentrations appear to be

present in youth with the metabolic syndrome [3]. Data is also present suggesting that some of these associations are dependent on adiposity levels [11, 14]. However, no study has demonstrated associations of resistin with BP or lipids in youth [16].

Although several studies have been conducted to date on this topic, most of the previous studies in children focused on a particular adipokine, mostly adiponectin. Moreover, given the interrelationship between insulin resistance and cardiovascular disease [1, 5, 7], it would be expected that adipokines related to insulin resistance are associated with other cardiovascular risk factors. The purpose of this study is twofold: first to evaluate the associations between different adipokines and BP and blood lipids, and second to evaluate if these associations are attenuated by overall or abdominal adiposity or dependent on weight status. We hypothesized that TNF- α , IL-6, and resistin would be positively associated with systolic blood pressure (SBP), TG, total cholesterol (TC), and negatively associated with high-density lipoprotein (HDL). In contrast, adiponectin would be negatively associated with SBP, TG, TC, and positively associated with HDL. Furthermore, these relationships would be exacerbated in overweight adolescents and attenuated by adiposity.

Subjects and Methods

Subjects

Data from 120 adolescents (60 girls and 60 boys), aged 10–14 years who participated in the Cardiovascular Health in Children and Youth (CHIC III) Study between 2000 and 2003, were utilized for this retrospective study. The CHIC studies have been multi-cohort studies, focused on cardiovascular risk factors in school-aged youth [17, 18]. All subjects signed an assent and their parents or guardians signed an informed consent approved by the institutional review board of the institution. Potential subjects for this analysis had to be in pubertal developmental stages 2–4 (mid-puberty) and have normal glucose concentrations (fasting glucose <5.6 mmol/l). All subjects meeting the prescribed criteria were included in the subject pool. Body mass index (BMI) percentiles by age and sex were used to classify youth into obese and normal weight categories [19]. BMI percentile was chosen because it is the standard clinical criteria in the USA for determination of weight status [19]. The 75th and 95th percentile thresholds were chosen to maximize differences in adiposity between the normal weight and overweight groups. From the subject pool, a subset was identified which included those with a BMI $\leq$ 75th percentile (normal weight) and those with BMI $\geq$ 95th percentile (overweight). From this subset 60 normal weight and 60 overweight subjects were randomly selected based on their sex using the select random cases procedure of the Statistical Package for Social Sciences for Windows version 9.0 (SPSS Inc., Chi-

cago, Ill., USA). 60 subjects per group were sufficient to detect group differences of 0.4 ng/ml for TNF- α , 0.6 ng/ml for IL-6, 2.1 ng/ml for resistin and 1.6 mg/ml for adiponectin, with an $\alpha = 0.05$, $\beta = 0.2$ and a power of 80%.

Anthropometric, Pubertal Status and BP Measurements

Measurements took place in the school setting within 3 days of blood sampling. The subjects were dressed in shorts and T-shirts without shoes. Height was measured to the nearest 0.1 cm using a stadiometer (Perspective Enterprises, Kalamazoo, Mich., USA) and body mass was obtained in kilograms to the nearest 0.1 kg using a Scale-Tronix electronic scale (model 5602; Scale-Tronix, Carol Stream, Ill., USA). BMI was computed using body mass in kilograms divided by height in meters squared ($\text{BMI} = \text{kg}/\text{m}^2$) and used as an indicator of overall adiposity. Waist circumference was measured in duplicate to the nearest 0.1 cm using a tape with tensiometer (Gulick Tape; Country Technologies Inc., Gays Mill, Wisc., USA) and was used as an indicator of abdominal adiposity. All measurements were obtained following procedures described by the National Health and Nutrition Examination Survey [20]. Puberty was determined using a validated self-completed questionnaire that assessed puberty based on growth spurt in height, pubic hair development, skin changes in both boys and girls, facial hair growth and voice change in boys only, and breast development and menarche in girls [21]. Ethnicity was self-reported.

After the anthropometric measures were obtained the subjects sat for 5 min before BP was measured using a random-zero mercury sphygmomanometer and appropriately sized cuff (Baum Inc., Copiague, N.Y., USA). BP readings were obtained in duplicate in the right arm following American Heart Association procedures. If the pressure readings differed by ≥ 10 mm Hg, a third reading was taken and the three readings averaged.

Blood Sampling and Analyses

Blood samples were obtained after an overnight fast, between 7 and 9 a.m., at the school, using sterile venipuncture technique. Fasting compliance was verbally verified by on-site research assistants. Blood samples were collected in tubes containing EDTA and immediately centrifuged to obtain plasma. Plasma was aliquoted, maintained in dry ice and transported to the laboratory where it was kept frozen at -80°C until analyzed. Concentrations of TC, HDL cholesterol and TG were tested on fasting plasma samples using automated coupled-enzymatic procedures and a Hitachi 911 analyzer (Boehringer-Mannheim Corp.) by a CLIA-approved clinical laboratory.

Concentrations of plasma of the adipokines resistin, TNF- α , IL-6, and adiponectin were measured using standard enzyme immunoassay procedures (Linco Research Inc., St. Charles, Mo., and R&D Systems, Minneapolis, Minn., USA). The resistin assay had a sensitivity of 0.5 ng/ml with intra- and inter-assay coefficients of variation of <10 and 11.2%, respectively. The adiponectin assay had a limit of sensitivity of 0.78 ng/ml with intra- and inter-assay coefficients of variation of <10 and 11.6%, respectively. The TNF- α kit had a sensitivity of 0.5 pg/ml while the IL-6 kit had a sensitivity of 0.2 pg/ml. The intra-assay coefficient of variation for TNF- α and IL-6 was $<10\%$. The inter-assay coefficient of variation for TNF- α was 12.2% and for IL-6 was 18.2%.

Table 1. Anthropometric and metabolic characteristics of subjects by weight status groups: mean $\pm$ SD (minimum–maximum)

	Normal weight (n = 60)	Overweight (n = 60)
Physical		
Female/male	30/30	30/30
Age, years	12.0 $\pm$ 1.0 (10–14)	12.0 $\pm$ 1.0 (11–14)
Puberty (stages 1–5)	2.9 $\pm$ 0.7 (2–4)	3.2 $\pm$ 0.7* (2–4)
Stature, cm	152.1 $\pm$ 7.6 (135.4–170.3)	159.8 $\pm$ 7.9* (144.7–186)
Body mass, kg	40.4 $\pm$ 5.9 (26.7–56.6)	76.3 $\pm$ 15.2* (52.4–123)
BMI	17.4 $\pm$ 1.4 (13.6–20.1)	29.7 $\pm$ 5.0* (23.7–46.4)
Waist, cm	61.7 $\pm$ 5.3 (52.3–80.2)	90.0 $\pm$ 10.5* (70.1–118.8)
CVR risk factors		
SBP, mm Hg	103 $\pm$ 10 (85–120)	113 $\pm$ 11* (93–143)
DBP, mm Hg	65 $\pm$ 11 (36–89)	66 $\pm$ 10 (43–99)
TC, mg/dl	154.6 $\pm$ 25.3 (102–258)	150.7 $\pm$ 29.2 (107–267)
HDL, mg/dl	52.5 $\pm$ 10.7 (34–87)	40.6 $\pm$ 8.3* (24–58)
TG, mg/dl	61.9 $\pm$ 25.8 (25–155)	86.7 $\pm$ 39.1* (27–189)
Adipokines		
Adiponectin, μ g/ml	12.5 $\pm$ 5.0 (4.4–30.4)	7.6 $\pm$ 3.4* (2.9–19.4)
Resistin, ng/ml	10.2 $\pm$ 4.0 (3.7–22.3)	11.3 $\pm$ 6.0 (4.4–41.3)
TNF- α , pg/ml	1.4 $\pm$ 0.7 (0.2–3.8)	1.9 $\pm$ 1.4* (0.3–7.2)
IL-6, pg/ml	1.5 $\pm$ 1.6 (0.1–6.4)	1.8 $\pm$ 2.0 (0.2–9.6)

* $p < 0.05$ for weight status groups (normal weight vs. overweight).

Statistical Analysis

For descriptive purposes, mean and standard deviations were computed for all variables by weight groups. Since the adipokines and lipid concentrations were not normally distributed, their values were transformed using the natural log. To determine the associations between SBP, lipids and each adipokine, analyses were conducted in three steps. First, we computed bivariate correlations between BP, lipids, and all adipokines. Second, for those associations that were statistically significant we tested several multiple regression models. The first model included the risk factor (SBP, TC, HDL, or TG) and one adipokine controlling for ethnicity (African-American, Caucasian and other), sex, age or puberty. Only control variables that were significantly associated with the dependent variable in this first model were kept in subsequent models. The second model was computed to evaluate if the association between the risk factor and the adipokine was attenuated by abdominal adiposity (waist circumference). The third model was computed to evaluate if the association was present depending on the weight status of the adolescents and included an interaction term between the adipokines and weight status. Weight status was based on the BMI percentile (≤ 75 th and ≥ 95 th percentile) indicating normal weight or obesity. Interaction terms not statistically significant were excluded from the models presented. Statistical significance was held at $p < 0.05$. Analyses were conducted using SPSS for Windows version 9.0.

Results

Subjects' Characteristics

The subjects' characteristics are presented in table 1 by weight groups. Racial distribution was 57% African-American, 37% Caucasian, and 6% other races including Native American, Asian and other. 29 participants were in pubertal stage 2, 53 were in pubertal stage 3, and 38 were in pubertal stage 4. The overweight group was taller and slightly advanced in pubertal status than the normal weight group. In addition, some cardiovascular risk factors and adipokine concentrations were significantly ($p < 0.05$) different between normal and overweight adolescents.

Bivariate Correlations in the Whole Cohort

SBP was significantly ($p < 0.05$) related to adiponectin ($r = -0.185$), resistin ($r = 0.207$), and IL-6 ($r = 0.238$). Diastolic blood pressure (DBP) was not related to any adipokine ($p > 0.05$). HDL was significantly ($p < 0.05$) associated with adiponectin ($r = 0.398$) and TNF- α ($r = -0.227$), while TG were only associated with adiponectin ($r = -0.292$; $p < 0.05$). No other significant associations were obtained.

Table 2A. Multivariate regression models for the relationships between SBP and adipokines controlling for ethnicity and puberty

Independent variable	β -Coefficient	p	Model R ²	p
Adiponectin	-0.195	0.028	0.152	0.001
Adiponectin	0.038	0.677		
Waist	0.463	0.000	0.308	0.000
Adiponectin	0.003	0.975		
Weight status	0.382	0.000	0.253	0.000
Resistin	0.147	0.110	0.152	0.002
Resistin	0.079	0.343		
Waist	0.426	0.000	0.308	0.000
Resistin	0.107	0.214		
Weight status	0.361	0.000	0.258	0.000
IL-6	0.246	0.008	0.170	0.000
IL-6	0.134	0.103		
Waist	0.420	0.000	0.330	0.000
IL-6	0.177	0.035		
Weight status	0.363	0.000	0.289	0.000

Significant models presented.
 Weight status coding: normal = 0 (BMI <75th percentile); overweight = 1 (BMI >95th percentile).
 All cytokines were logarithmically transformed.

Multiple Regression Analyses

Regression analyses were conducted only for SBP, HDL, TG and the adipokines that were significantly related to them in the bivariate analyses. For each parameter, several separate regression models were tested; initially all control variables were included in the first model but only significant ones were kept in subsequent models. All regression models for SBP included puberty and ethnicity as control variables. HDL and TG models did not include control variables. The standardized β -coefficients $\pm$ SE are presented for each adipokine as well as the total R² $\pm$ SE for the regression model. For SBP, the models containing adiponectin, resistin and IL-6 were significant (table 2A). Waist circumference attenuated all associations ($p < 0.001$) and so did weight status ($p < 0.001$). HDL was significantly associated with adiponectin, presenting a significant interaction term between *adiponectin and weight status* ($p = 0.033$). HDL was negatively associated with TNF- α ($p = 0.021$), but the association became attenuated when waist circumference or weight status were included in the model ($p = 0.087$ and $p = 0.229$, respectively). TG were also associated with adiponectin, but the weight status interaction

Table 2B. Multivariate regression models for the relationships between HDL and TG, with adiponectin, and TNF- α

Independent variable	β -Coefficient	p	Model R ²	p
HDL models				
Adiponectin	0.390	0.000	0.152	0.000
Adiponectin	0.146	0.097		
Waist	-0.489	0.000	0.332	0.000
Adiponectin	0.339	0.009		
Weight status	0.434	0.312		
Adiponectin and weight status	-0.830	0.033	0.336	0.000
TNF- α	-0.216	0.021	0.046	0.021
TNF- α	-0.136	0.087		
Waist	-0.535	0.000	0.327	0.000
TNF- α	-0.099	0.229		
Weight status	-0.511	0.000	0.294	0.000
Triglyceride model				
Adiponectin	-0.350	0.000	0.176	0.000
Adiponectin	-0.142	0.162		
Weight status	0.282	0.006	0.142	0.000
Adiponectin	-0.119	0.224		
Waist	0.346	0.001	0.175	0.000

was not significant ($p > 0.05$). Both waist circumference and weight status attenuated this last association (table 2B).

Discussion

We evaluated the associations between BP and lipids and 4 adipokines. SBP was associated with adiponectin, resistin and IL-6. HDL was related to adiponectin and TNF- α , while TG were only associated with adiponectin. DBP and TC were not significantly associated with any adipokine in the participating youth. The associations obtained in our study were significant but not very strong. Since stronger associations have been reported in adults [15, 22–24], the weak associations may be simply due to the fact that adolescents have not had sufficient time to fully develop the relationships.

Some research has shown that increased adiponectin is associated with lower SBPs [13, 25]. In vitro, adiponectin reduces monocyte adhesion in endothelial cells, preventing the development of lesions in the endothelium [10]. It also prevents increases in the intima media thick-

ness modulating smooth muscle proliferation [26]. Therefore, the negative associations between SBP and this adipokine are not surprising. The positive associations between SBP and resistin and IL-6 are novel findings in youth. Gilardini et al. [27] failed to demonstrate an association between ambulatory SBP and IL-6 in obese young children. However, the positive association found in the present study appears reasonable since studies in youth with obesity of varying degrees and metabolic syndrome have shown elevations of IL-6 concentrations compared to normal weight youth [3].

Moon et al. [14] showed a positive association of TNF- α with BP in older obese adolescents, but we did not. TNF- α appears to suppress the release of nitric oxide (a vasodilator), therefore increasing total peripheral resistance and BP [28]. Our sample included both normal weight and overweight younger adolescents. Perhaps in obese youth this association manifests itself with increasing age.

The overweight adolescents had higher circulating concentrations of TG and low levels of HDL than the normal weight adolescents. Concomitantly, we found a positive association between HDL and adiponectin and a negative association between TG and adiponectin. These results support previous findings by Huang et al. [29] and Bacha et al. [25]. Interestingly, the association between adiponectin and HDL was stronger in normal weight adolescents, perhaps because these adolescents had higher HDL concentrations as well as higher adiponectin concentrations. The negative association between adiponectin and TG could potentially be related to the fact that adiponectin has been shown to inhibit TG production by the liver [8].

Van Gaal et al. [5] suggested that visceral fat production of IL-6 and TNF- α , as part of the inflammatory process, affects liver production of lipids. TNF- α is associated with increased TG production, and a decrease in lipoprotein lipase [30]. IL-6 in turn stimulates the release of very-low-density lipoproteins which can become oxidized to low-density lipoprotein. Moon et al. [14] showed a positive association between TNF- α and TG in obese adolescents and we showed a negative association with HDL further expanding on the relationship between TNF- α and dyslipidemia.

To our knowledge, no other studies have shown associations between lipids and resistin in youth. However, in adults, resistin was positively associated with TG and negatively with HDL concentrations [24]. In mice, resistin has been related to increased hepatic lipogenesis and very-low-density lipoproteins export [31]. Because the

average values of lipids are lower in youth than adults [32], it is possible that the relationship between resistin and lipids becomes evident at older ages when dyslipidemia is more prevalent. The relationship between lipids and IL-6, in particular between IL-6 and TG, has only been recently shown in obese adolescents but not in normal weight or overweight adolescents [33]. Interestingly, previous studies in adults did not show such an association [22, 23]. Probably since our study included normal weight and obese adolescents, we did not detect this association either.

We and others have previously demonstrated small (~20%) differences in these adipokine concentrations between overweight and normal weight youth [3, 14, 29, 34]. Therefore, as our data suggest, the associations between these adipokines and cardiovascular risk factors in youth are dependent on adiposity, as associations were attenuated when including adiposity surrogates in the regression models. We also know that BP is related to stature as well as adiposity and our overweight group was taller than our normal weight group (table 1). Our data, not reported here, suggests that the relationship between BP and weight is stronger than the relationship between BP and height. Furthermore, there is strong collinearity between height and weight; thus we chose not to adjust our BP analysis for height.

The adipose tissue is one source of all these adipokines [6, 28, 35, 36] and adiponectin is produced solely by the adipocyte. In contrast, TNF- α , IL-6 and resistin are also produced by the muscle and immune system cells [6, 28, 35]. Since the prevalence of cardiovascular risk factors is higher with increased adiposity, the associations between this particular adipokine and risk factors is perhaps more consistent [11, 25, 29]. A potential hypothesis is that adipokines explain part of the inter-individual variability of BP or blood lipids not already explained by adiposity (BMI or waist circumference). Although our data does not allow us to test this hypothesis, we can suggest that in youth any influence of the adipokines is, in most cases, dependent on adiposity.

This study was conducted in previously collected blood samples. There was no control in the day-to-day variation in adipokine concentrations, however they were within ranges of previously published concentrations in youth [3, 12, 14, 25, 29]. The even sample size of normal weight and overweight subjects allowed inferences on the role of weight status in the studied associations. However, this sample characteristic may have limited the results with respect to the cardiovascular risk factors, as their prevalence increases with increased adiposity.

Conclusion

The understanding of the roles of these adipokines is still a work in progress. We extended the knowledge by showing associations not only between adiponectin and BP or lipids, but also resistin, TNF- α and IL-6. SBP was related to most of the adipokines evaluated in this study. Higher SBP was associated with higher resistin and IL-6 concentrations and lower adiponectin concentrations. Higher HDL was associated with lower TNF- α and higher adiponectin concentrations while lower TG were associated with higher adiponectin concentrations. Neither

DBP nor TC were related to any of the adipokines. The available evidence suggests that increased adiposity mediates these associations between adipokines and cardiovascular risk factors during adolescence as most associations were attenuated by waist circumference or weight status.

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