INFLAMMATION, INSULIN, AND ENDOTHELIAL FUNCTION IN OVERWEIGHT CHILDREN AND ADOLESCENTS: THE ROLE OF EXERCISE

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Objectives To assess subclinical inflammation, fasting insulin, and endothelial function before and after exercise in overweight children and adolescents.

Study design Twenty-five children (body mass index [BMI] >85th percentile) were assessed for brachial artery flow-mediated dilation (FMD), nitroglycerin-induced dilation, C-reactive protein (CRP), lipids, glucose, insulin, oral glucose tolerance, body composition, aerobic fitness (peak oxygen uptake [VO_2peak]), and blood pressure. Twenty of these persons were equally and randomly assigned to either 8 weeks of stationary cycling or to a non-exercising control group.

Results A baseline correlation was found between CRP and fasting insulin ($r = 0.62$; $P < .001$), which remained significant after adjusting for baseline variables ($r = 0.53$; $P < .05$). After 8 weeks, significant improvements were observed in the exercise group compared with the control group for VO_2peak (exercise group = 21.8 ± 2.1 to 23.2 ± 1.5 mL/kg/minute vs control group = 23.4 ± 1.6 to 20.9 ± 2.2 mL/kg/minute; $P < .05$), high-density lipoprotein (HDL) cholesterol (exercise group = 1.02 ± 0.03 to 1.10 ± 0.04 mmol/L vs control group = 1.08 ± 0.07 to 0.99 ± 0.09 mmol/L; $P < .05$), and FMD area under the curve (AUC) (exercise group = 746 ± 66 to 919 ± 94 %•sec vs control group = 731 ± 102 to 515 ± 73 %•sec; $P < .05$).

Conclusions In overweight children and adolescents, CRP is independently associated with fasting insulin. Eight weeks of aerobic exercise improves fitness, HDL cholesterol, and endothelial function in this group. *(J Pediatr 2004;145:731-6)*

The prevalence of overweight and obese children is increasing.[1] Currently, more than 22% of all children in the United States are overweight.[2] Recent data have shown that obesity is independently associated with coronary atherosclerosis in young adults.[3] The initial development of cardiovascular disease likely occurs during childhood,[4] and obesity may accelerate this process.

Obesity, inflammation, and insulin resistance often occur in concert, and there is evidence to suggest that inflammation and insulin resistance may correlate independent of obesity. In healthy adults, C-reactive protein (CRP) has been shown to be significantly related to fasting insulin and the homeostasis model assessment for insulin resistance, even after adjustment for measures of obesity.[5,6,7] Although these associations have yet to be shown in children, evidence exists that adiposity in children is related to elevated CRP levels[8,9,10] and insulin resistance.[11]

Both inflammation and insulin resistance may mediate, or further contribute to, endothelial dysfunction.[5] A recent study has demonstrated that obese but otherwise healthy children exhibit vascular endothelial dysfunction and increased arterial stiffness compared with their normal-weight peers.[12] These findings are important because endothelial dysfunction occurs very early in the pathogenesis of cardiovascular disease and is considered an initial marker of atherosclerosis.

The major factor potentially linking obesity, inflammation, insulin resistance, and endothelial dysfunction is sedentary behavior. Studies in adults have consistently shown

ANOVA	Analysis of variance	FMD	Flow-mediated dilation
AUC	Area under the curve	HDL	High-density lipoprotein
BMI	Body mass index	LDL	Low-density lipoprotein
CRP	C-reactive protein	SBP	Systolic blood pressure
DBP	Diastolic blood pressure	VO_2peak	Peak oxygen uptake

See editorial, p 719.

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Supported in part by Minnesota Obesity Center Grant #: 1 P30 DK 50456-08 (D.R.K.), American Heart Association Pre-Doctoral Grant #: 0315213Z (A.S.K.), and GCRC: M01-RR00400, General Clinical Research Center Program, NCRR/NIH.

Data presented in this manuscript appear in a dissertation titled ''The effects of aerobic exercise training on vascular structure and function in obese children'' that was completed at the University of Minnesota in February 2004.

Submitted for publication Mar 31, 2004; last revision received Jun 28, 2004; accepted Aug 3, 2004.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2004.08.004

improvements in endothelial function with aerobic exercise training,[13,14,15] however data are lacking in children. Therefore, the purpose of this study was to assess inflammation, fasting insulin as a marker of insulin resistance, and endothelial function in overweight children and adolescents and to determine the effects of aerobic exercise training on these variables.

METHODS

Subjects

Twenty-five healthy, overweight children and adolescents (body mass index [BMI] >85th percentile for age and gender) were recruited from the greater Minneapolis/St. Paul, Minnesota, metropolitan area to participate in this study. Baseline characteristics for all participants are provided in Table I. Following baseline testing, 20 of the subjects were randomized to either the exercise or the control group. All participants and parents/guardians gave written informed assent and consent. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board.

Vascular Protocol

Participants arrived fasted in the morning at the University of Minnesota General Clinical Research Center. After 15 minutes of quiet rest, vital signs were collected followed by assessment of flow-mediated dilation (FMD) of the brachial artery as previously described in our lab and others.[16,17] Briefly, standard ultrasonography equipment (Image Point Hx, Philips Medical, Bothell, Wash) with a 7.5-MHz linear array probe was used to obtain B-mode images of the left brachial artery (approximately 2-10 cm proximal to the elbow). Following measurement of resting artery diameter, a blood pressure cuff was inflated just below the elbow (distal to imaged artery segment) to a pressure of 200 mm Hg for 5 minutes. Brachial artery diameter was measured continuously for a total of 3 minutes after cuff release to determine peak percent dilation (FMD peak), based on initial artery diameter, and FMD area under the curve (AUC). After generating percent dilation curves over a 3-minute period following cuff release, the AUC was calculated in a statistical program (GraphPad Prism version 4.0, GraphPad Software, Inc, San Diego, Calf) for subsequent analysis. Following the measurement of FMD, the brachial artery was imaged 3 minutes after the administration of 0.3 mg sublingual nitroglycerin in order to assess endothelium-independent dilation. Images were captured and triggered off of the R wave of the electrocardiogram (end-diastolic diameter) and then were digitized and stored on a personal computer for later off-line analysis. Electronic wall-tracking software (CVI, Information Integrity, Boston, Mass) was used for analysis. All digital files were analyzed by the same trained reader and were coded to allow for blinded analysis of all FMD data. Reproducibility for the FMD technique in our lab has shown a mean difference of 0.53% ± 0.28% for analyses separated by 1 week in 10 young, healthy persons (unpublished data).

Laboratory Measurements

Blood was collected for the measurement of lipids, ultrasensitive CRP, glucose, and insulin. None of the subjects had been ill or injured in the previous 2 weeks. Assays were conducted at Fairview Diagnostic Laboratories, Fairview-University Medical Center, Minneapolis, Minn. Cholesterol, triglycerides, and glucose were determined by colorimetric reflectance spectrophotometry. CRP was analyzed with an ultra-sensitive assay utilizing rate nephelometry. Insulin was determined by chemiluminescent immunoassay. Standard 2-hour oral glucose tolerance tests were performed, and 2-hour blood glucose values were reported. Dietary recall questionnaires were administered by a registered dietician to estimate daily kilocalorie consumption. Body composition was determined by dual-energy x-ray absorptiometry. Tanner growth stage for pubertal development was determined by a trained pediatrician. Aerobic fitness was quantified as peak oxygen uptake (VO_2peak) and was assessed with a graded intensity protocol on a stationary cycle ergometer starting at 20 W and increasing 20 W every 2 minutes until exhaustion. Expired oxygen and carbon dioxide concentrations and volumes were collected and analyzed using a MedGraphics CPX-D metabolic cart (MedGraphics, St. Paul, Minn).

Exercise Protocol

Controls did not participate in structured exercise and were instructed to maintain current levels of physical activity. Exercise training was conducted in the Laboratory of Physiological Hygiene and Exercise Science at the University of Minnesota. All exercise training was supervised, occurred 4 times per week, and consisted of stationary cycling starting at 50% to 60% of VO_2peak for 30 minutes per session (included 5-minute warm-up and cool-down). The heart rate corresponding to the appropriate percentage of VO_2peak was used to monitor exercise intensity. Every week, either the intensity or the duration was increased until subjects were exercising at 70% to 80% VO_2peak for durations of 50 minutes during the last 2 weeks.

Statistical Analyses

Results are expressed as mean ± SEM. CRP and triglycerides were log-transformed for all analyses. Pearson's correlation coefficients were calculated. Statistical adjustments for percent body fat, lipids, fasting glucose, systolic blood pressure (SBP), and diastolic blood pressure (DBP) (confounding variables) were carried out with a regression model using the partial correlation coefficient feature of the Statistical Package for the Social Sciences version 11.0 for Windows (SPSS, Inc, Chicago, Ill). For the exercise data analysis, unpaired t tests were used to compare baseline variables between groups. Comparison of variables between groups before and after 8 weeks was analyzed by two-way repeated measures analysis of variance (ANOVA) with Bonferroni post-hoc tests where appropriate. An α value of .05 was used to signify statistical significance. Statistical

Table I. Baseline characteristics of overweight children and adolescents (n = 25)

Age (y)	10.9 ± 0.4
Gender (M/F)	12/13
Tanner growth stage	2.0 ± 0.2
Height (cm)	151.4 ± 2.3
Weight (kg)	71.0 ± 4.6
BMI (kg/m^2)	30.4 ± 1.3
Body fat (%)	44.1 ± 1.3
SBP (mm Hg)	122.4 ± 3.1
DBP (mm Hg)	66.2 ± 1.9
Total cholesterol (mmol/L)	4.01 ± 0.15
LDL cholesterol (mmol/L)	2.50 ± 0.14
HDL cholesterol (mmol/L)	1.06 ± 0.03
Triglycerides (mmol/L)	0.99 ± 0.11
Fasting glucose (mmol/L)	4.71 ± 0.06
Fasting insulin (pmol/L)	76.8 ± 8.9
C-reactive protein (mg/L)	4.2 ± 0.8

Data are presented as mean ± SEM.

analyses for all exercise data were performed with GraphPad Prism version 4.0.

Results

At baseline, 11 participants (44%) were classified with the metabolic syndrome according to modified pediatric criteria.[18] Significant correlations were found between fasting insulin and percent body fat (r = 0.44; P = .03), CRP and percent body fat (r = 0.64; P = .001), and CRP and fasting insulin (r = 0.62; P = .001; Figure 1). After adjusting for percent body fat, lipids, fasting glucose, SBP, and DBP, the relationship remained significant for CRP and fasting insulin (r = 0.53; P = .03).

Complete descriptive and clinical characteristics before and after the 8-week intervention period are presented in Tables II and III, respectively. There were no statistically significant differences between groups for any of the baseline variables, including FMD AUC (Figure 2). No significant differences between groups were observed over 8 weeks for body weight, BMI, percent body fat, SBP, DBP, total cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, glucose, insulin, glucose tolerance, CRP, resting brachial diameter, FMD peak, nitroglycerin dilation, and kilocalories consumed per day. Within group analyses showed that both groups significantly increased in height over the 8-week period; however, no differences were found between the groups. VO_2peak significantly decreased within the control group and increased in the exercise group compared with the control group. HDL cholesterol significantly improved in the exercise compared with the control group. Compared with the control group, the exercise group significantly improved FMD AUC after 8 weeks of exercise (Figure 3). Nonsignificant trends toward improvement were observed in the exercise versus control group for fasting insulin (P = .09) and FMD peak (P = .09).

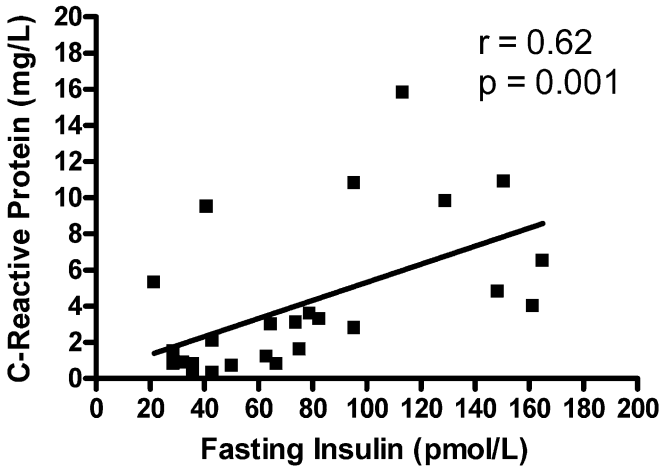

Figure 1. Relationship between fasting insulin and circulating C-reactive protein levels in overweight children and adolescents. The relationship between insulin and CRP remained significant after adjusting for baseline variables.

DISCUSSION

The baseline association between CRP and fasting insulin is consistent with previous work in adults showing an independent association between chronic, subclinical inflammation and markers of insulin resistance.[5,7] Data from the present study confirm these previous findings in adults and extend the associations between inflammation and fasting insulin, a marker of insulin resistance, to children.

Children make a unique and valuable model for assessing the complex relationship between inflammation and insulin resistance. Adult populations often present a clustering of potential confounding variables including age, smoking, hypertension, hyperlipidemia, and diabetes, which can hamper efforts to identify initial triggers for atherosclerosis and insulin resistance. This clustering of risk factors is more likely to be absent from a young and relatively healthy population, which allows for a more refined examination of these relationships. The present results suggest that early in life in overweight persons, inflammation is associated with fasting insulin levels and may be involved in the pathogenesis of insulin resistance. Because many inflammatory cytokines are stimulated by adipose tissue,[5] this may be an important mechanism by which obesity is associated with insulin resistance. Further work is needed to appropriately examine this hypothesis as these data were observed in a relatively small sample of persons.

The finding that 8 weeks of aerobic exercise training improves arterial endothelial function in overweight children and adolescents is important because of the need to identify nonpharmacological interventions aimed at improving endothelial function in young individuals. Three recently published studies have shown that short-term exercise in overweight and obese children and adolescents improves endothelial function.[19,20,21] Although our overall conclusions are similar to these studies, some important differences exist. First, we utilized stationary cycling as the exercise mode, whereas the

Table II. Subject descriptive characteristics before and after 8 weeks (n = 20)

	Exercise group (n=10)		Control group (n=10)	
Variable	**Before**	**After**	**Before**	**After**
Age (y)	11.0 ± 0.63	NA	11.0 ± 0.71	NA
Sex	M = 5, F = 5	NA	M = 4, F = 6	NA
Tanner growth stage	2.0 ± 0.33	NA	2.3 ± 0.42	NA
Height (cm)	152.8 ± 3.8	154.0 ± 3.7*	152.7 ± 3.8	153.9 ± 3.9*
Weight (kg)	75.4 ± 6.9	76.5 ± 7.2	73.5 ± 8.8	74.3 ± 8.9
BMI (kg/m^2)	32.1 ± 2.4	32.1 ± 2.5	30.5 ± 2.3	30.4 ± 2.2
Body fat (%)	45.1 ± 2.0	45.4 ± 2.1	44.6 ± 2.5	45.3 ± 2.4
VO_2peak (mL/kg/min)	21.8 ± 2.1	23.2 ± 1.5†	23.4 ± 1.6	20.9 ± 2.2*
Kcal consumed/d	3001 ± 255	2517 ± 214	3038 ± 329	2974 ± 359

Data are presented as mean ± SEM.
*$P < .05$ for within-group analysis.
†$P < .05$ for comparison between groups (ANOVA interaction term).

Table III. Subject clinical characteristics before and after 8 weeks (n = 20)

	Exercise group (n = 10)		Control group (n = 10)	
Variable	**Before**	**After**	**Before**	**After**
SBP (mm Hg)	125 ± 7	120 ± 2	124 ± 4	122 ± 5
DBP (mm Hg)	68 ± 4	65 ± 2	66 ± 2	68 ± 3
Total cholesterol (mmol/L)	3.83 ± 0.25	4.01 ± 0.22	4.02 ± 0.24	3.92 ± 0.26
HDL cholesterol (mmol/L)	1.02 ± 0.03	1.10 ± 0.04†	1.08 ± 0.07	0.99 ± 0.09
LDL cholesterol (mmol/L)	2.33 ± 0.26	2.36 ± 0.24	2.48 ± 0.19	2.32 ± 0.23
Triglycerides (mmol/L)	1.04 ± 0.17	1.22 ± 0.17	1.00 ± 0.21	1.32 ± 0.22
Fasting glucose (mmol/L)	4.64 ± 0.11	4.66 ± 0.08	4.73 ± 0.08	4.80 ± 0.15
Fasting insulin (pmol/L)	88.8 ± 16.5	66.7 ± 9.5	81.4 ± 12.7	84.7 ± 14.1
2-h glucose (mmol/L)	6.17 ± 0.58	6.49 ± 0.66	6.03 ± 0.43	5.86 ± 0.34
C-reactive protein (mg/L)	4.4 ± 1.6	4.8 ± 2.6	5.0 ± 1.2	3.8 ± 0.9
Brachial diameter (mm)	3.16 ± 0.14	3.10 ± 0.16	3.06 ± 0.15	2.99 ± 0.14
FMD peak (%)	6.8 ± 0.5	7.9 ± 0.7	6.8 ± 0.7	6.1 ± 0.6
FMD AUC (%•sec)	746 ± 66	919 ± 94†	731 ± 102	515 ± 73
Nitroglycerin dilation (%)	16.5 ± 1.5	21.3 ± 3.9	20.7 ± 1.9	24.1 ± 1.9

Data are presented as mean ± SEM.
*$P < .05$ for within-group analysis.
†$P < .05$ for comparison between groups (ANOVA interaction term).

above-referenced studies performed circuit and whole body exercise regimens. Combined, these data show that a wide variety of types of physical activity can improve arterial health in this group. Second, we measured VO_2peak to quantify fitness level before and after the study period in the control and exercise groups. We believe that VO_2peak is a more accurate measure of changes in fitness level compared with submaximal exercise testing. Lastly, similar to Woo et al,[19] we utilized nitroglycerin to assess endothelium-independent dilation of the brachial artery in overweight children following exercise training. This technique is important in that it distinguishes between changes in endothelial-derived nitric oxide versus changes in smooth muscle sensitivity in the vasculature.

In the current study, we did not include normal-weight children as controls to determine whether overweight children in our study exhibited endothelial dysfunction at baseline. However, the values for FMD peak in our data set were similar to those of the overweight children in the study by Tounian et al, which showed decreased FMD in this group compared with normal weight controls.[12] It is therefore reasonable to assume that overweight children in the present study had at least mild endothelial dysfunction. It is encouraging to observe significant improvements in arterial health in a time period as short as 8 weeks in this group. Moreover, in this study improvements in endothelial function of the brachial artery were observed even though training occurred mainly in the

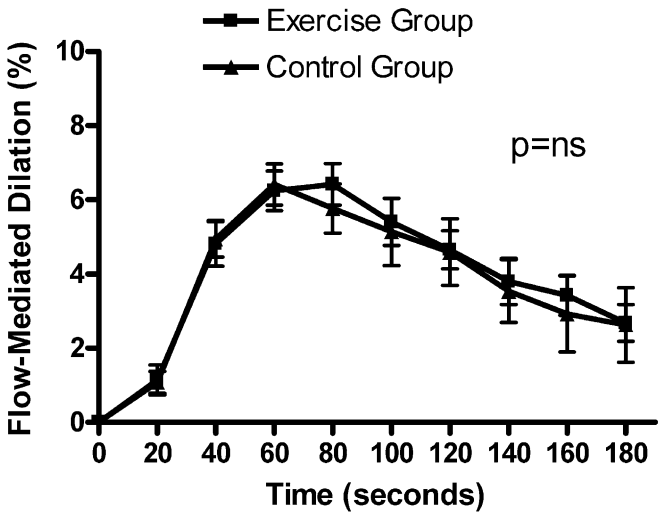

Figure 2. Comparison of baseline flow-mediated dilation between the exercise (square) and control (triangle) groups.

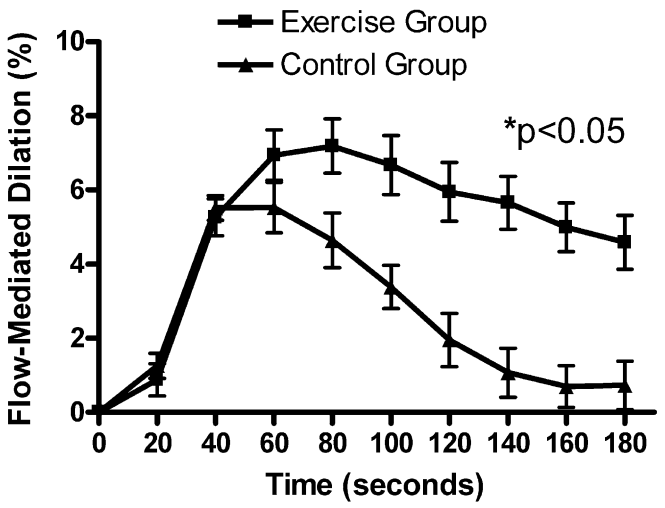

Figure 3. Comparison of flow-mediated dilation after 8 weeks in the exercise (square) and control (triangle) groups. Eight weeks of exercise significantly improved flow-mediated dilation.

lower limbs, suggesting systemic improvements in vascular health. We believe this to be a significant finding because the brachial artery is sometimes used as a surrogate vascular bed for coronary artery endothelial function.

Despite clear improvements in endothelial function, minimal changes in the traditional cardiovascular risk factor profile occurred, suggesting that exercise may have a direct beneficial role on the health of the vasculature. Particularly of interest in this group is that body weight and body composition remained the same after exercise, yet improvements in endothelial function still occurred. Recent data provided by Green et al[22] support this contention that at least short-term exercise has an independent effect on arterial health. A pooled analysis of previous exercise training studies in a broad range of populations characterized by endothelial dysfunction showed improvements in conduit and resistance vessel endothelial function without concomitant improvements in other cardiovascular risk factors.[32]

Mechanisms have been proposed to explain the independent beneficial effects of exercise on the integrity and health of the endothelium. One such mechanism is an increase in endothelial cell nitric oxide synthase gene expression following exercise training resulting from increased vascular wall shear stress via blood flow.[23] The upregulation of this gene stimulates increased production and bioavailability of nitric oxide leading to improved endothelial function. Although not measured directly in this study, it is reasonable to assume that systemic blood flow increased during exercise training leading to increased nitric oxide production through this pathway.

Also of interest is the fact that although fasting insulin displayed a trend toward improvement with exercise, no changes in CRP were observed. Because these two variables showed a significant independent correlation at baseline, it could be hypothesized that both would show improvements following exercise training. However, we found no correlation between improvements in fasting insulin and changes in CRP after exercise training. It may be that concomitant weight loss with exercise is necessary in order to stimulate improvements in the CRP profile, since adipocytes partially mediate the inflammatory response.[5] Alternatively, factors such as exercise training duration, intensity, and mode may differentially affect CRP levels and our protocol may not have been appropriate to elicit a change in the inflammatory profile. Finally, the limitation of sample size in this study may be another factor explaining our failure to observe changes in CRP, and a correlation with fasting insulin following exercise.

One aspect of our FMD protocol warrants brief discussion. In our laboratory we have the ability to quantify the total dilation response over a period of 3 minutes after cuff release. Jarvisalo et al[24] have previously reported the utility of this method and have described that using an arbitrary time point to assess FMD might not be appropriate in children because of the large variability in the time course of FMD in this group. Therefore, we believe that FMD AUC is an appropriate means by which to assess the overall response of the brachial artery to increased flow because it addresses diameter changes over a period of time, rather than at an isolated time point.

Although it is possible that the differences observed between the control and exercise groups over 8 weeks occurred because of random variation resulting from a relatively small sample size, we do not believe this to be the case. This study was conducted during the summer months while children were not in school. Experimental (ie, decrease in VO_2peak) and anecdotal (conversations with children and parents) observations have led us to believe that the control group engaged in less physical activity during these summer months compared with during the school year. Therefore, it is not surprising that VO_2peak decreased significantly and HDL cholesterol and FMD decreased nonsignificantly in the control group. We do not believe that the large differences in FMD over 8 weeks between the groups were entirely a result of measurement variability or random variation but

rather mainly a result of physiological changes that occurred from short-term behavior changes in the control group during the summer months (ie, decrease in physical activity).

Further work should be initiated utilizing larger samples of overweight children to assess the complex relationships between adiposity, inflammation, insulin resistance, and endothelial dysfunction. Additionally, future work should assess the long-term cardiovascular effects of exercise training in overweight children and examine strategies to implement this behavior modification.

REFERENCES

1. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. Br Med Bull 1997;53:238-52.

2. Goran MI. Metabolic prescursors and effects of obesity in children: a decade of progress, 1990-1999. Am J Clin Nutr 2000;73:158-71.

3. McGill HC, McMahan CA, Herderick EE, Zieske AW, Malcom GT, Tracy RE, et al. Obesity accelerates the progression of coronary atherosclerosis in young men. Circulation 2002;105:2712-8.

4. Berenson GS, Srinivasan SR, Bao W, Newman III WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. N Engl J Med 1998;338:1650-6.

5. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.

6. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes 2001;25:1416-20.

7. Pradhan AD, Cook NR, Buring JE, Manson JE, Ridker PM. C-reactive protein is independently associated with fasting insulin in nondiabetic women. Arterioscler Thromb Vasc Biol 2003;23:650-5.

8. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics 2001; 107:E13.

9. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. J Pediatr 2001;138:486-92.

10. Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, et al. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. Atherosclerosis 2000;149:139-50.

11. Sinaiko AR, Jacobs DR, Steinberger J, Moran A, Luepker R, Rocchini AP, et al. Insulin resistance syndrome in childhood: associations of the euglycemic insulin clamp and fasting insulin with fatness and other risk factors. J Pediatr 2001;139:700-7.

12. Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet 2001;358:1400-4.

13. DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 2000;102:1351-7.

14. Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, et al. Exercise training enhances endothelial function in young men. J Am Coll Cardiol 1999;33:1379-85.

15. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, et al. Effect of exercise on coronary endothelial function in patients with coronary artery disease. N Engl J Med 2000;342:454-60.

16. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk for atherosclerosis. Lancet 1992;340:1111-5.

17. Kaiser DR, Billups K, Mason C, Wetterling R, Lundberg JL, Bank AJ. Impaired brachial artery endothelium-dependent and -independent vasodilation in men with erectile dysfunction and no other clinical cardiovascular disease. J Am Coll Cardiol 2004;43:179-84.

18. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med 2003;157:821-7.

19. Woo KS, Chook P, Yu CW, Sung RYT, Qiao M, Leung SSF, Lam CWK, Metreweli C, Celermajer DS. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation 2004;109:1981-6.

20. Watts K, Beye P, Siafarikas A, O'Driscoll G, Jones TW, Davis EA, et al. Effects of exercise training on vascular function in obese children. J Pediatr 2004;144:620-5.

21. Watts K, Beye P, Siafarikas A, Davis EA, Jones TW, O'Driscoll G, et al. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. J Am Coll Cardiol 2004;43:1823-7.

22. Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR, O'Driscoll JG. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. Am J Physiol Heart Circ Physiol 2003;285:H2679-87.

23. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Cir Res 1994;74: 349-53.

24. Jarvisalo MJ, Ronnemaa T, Volanen I, Kaitosaari T, Kallio K, Hartiala JJ, et al. Brachial artery dilatation responses in healthy children and adolescents. Am J Physiol Heart Circ Physiol 2002;282:H87-92.