Effects of physical training and its cessation on the hemostatic system of obese children[1–3]

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ABSTRACT

Background: Physical training can improve hemostatic function in adults, thereby reducing heart disease risk, but no information is available in children on whether physical training can enhance hemostatic function.

Objective: The purpose of this investigation was to examine the effects of a physical training program on hemostatic variables in a biethnic group of obese children.

Design: Children were randomly assigned to 2 groups. Group 1 participated in physical training for 4 mo and then ceased physical training for 4 mo, whereas group 2 did no physical training for the first 4 mo and then participated in physical training for 4 mo. Plasma hemostatic variables [fibrinogen, plasminogen activator inhibitor 1 (PAI-1), and D-dimer) were measured at months 0, 4, and 8.

Results: Analyses of variance revealed no significant group-by-time interactions for the hemostatic variables. When data from both groups were combined there was a significant decrease in D-dimer after 4 mo of physical training ($P < 0.05$). Factors explaining individual differences in responsiveness to the physical training revealed that individuals with greater percentage fat before physical training showed greater reductions in fibrinogen and D-dimer, and that blacks showed greater reductions in D-dimer than whites ($P < 0.05$). Stepwise multiple linear regression showed that only higher prephysical training concentrations of fibrinogen, PAI-1, and D-dimer explained significant proportions of the variation in changes in these variables.

Conclusions: In obese children, 4-mo periods of physical training did not lead to significant changes in hemostatic variables. Children with greater adiposity and concentrations of hemostatic factors before physical training showed greater reductions in hemostatic variables after physical training than did children with lesser values. *Am J Clin Nutr* 1999;69:1130–4.

KEY WORDS Hemostatic factors, D-dimer, fibrinogen, physical training, exercise, children, obesity, cardiovascular disease, plasminogen activator inhibitor 1, PAI-1

INTRODUCTION

In adults, several hemostatic factors are associated with some unfavorable aspects of cardiovascular health (1–3). Fibrinogen, an acute phase reactant synthesized in the liver, influences blood viscosity through red blood cell aggregation (4), with high concentrations being strong predictors of ischemic heart disease (5). Plasminogen activator inhibitor 1 (PAI-1), a glycoprotein involved with tissue-type plasminogen activator inhibition, may contribute to atherosclerosis (6) by limiting endogenous fibrinolysis (7). Obesity is a metabolic disorder that is associated with increased PAI-1 concentrations (8). D-Dimer, an end product of plasmin digestion of cross-linked fibrin and a direct marker of ongoing fibrinolysis (3), is associated with future myocardial infarction risk (9) and high concentrations have been observed in individuals with atherosclerosis (3). In cross-sectional surveys of children, body fatness has been found to be associated with D-dimer concentrations (10).

In studies in adults, physical training has proven to be one nonpharmacologic way to improve hemostatic function and reduce cardiovascular disease risk (11). To date, no studies have examined the effects of physical training on hemostatic function in children. Therefore, the purpose of this investigation was to examine the effects of a controlled physical training program on hemostatic variables in a biethnic group of obese children. A second purpose was to explore the factors associated with individual differences in responses of the hemostatic system to physical training.

SUBJECTS AND METHODS

Subjects

Subjects were 43 apparently healthy obese children aged 7–11 y, who were above the 85th percentile in triceps skinfold thickness for sex, ethnicity, and age (12). Subjects were not involved in other weight-control or exercise programs, and were not restricted in their physical activity. Children were recruited

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by using promotional flyers distributed through selected public and private schools in Augusta, GA. To facilitate transportation, schools were selected on the basis of their proximity to our physical training center. Children and their parents attended an orientation session and gave informed consent in accordance with procedures of the Human Assurance Committee of the Medical College of Georgia.

Testing sessions were conducted at baseline, after 4 mo, and after 8 mo of the experiment. The baseline characteristics of the subjects are shown in **Table 1**. After baseline testing, children were randomly assigned, within sex and ethnicity, to group 1 or group 2. Group 1 engaged in physical training for the first 4-mo period and then ceased formal physical training for the next 4 mo, whereas group 2 did no physical training for the first 4 mo and then engaged in physical training for the next 4 mo. Baseline testing was completed by 43 children, 40 completed the 4-mo testing, and 38 completed the 8-mo testing. Five children withdrew from the study because of after-school conflicts or relocation of parents.

Anthropometry

Weight was assessed with a balance scale and height with a stadiometer. Body mass index (BMI) was calculated by dividing body weight (kg) by height $(m)^2$. Total body composition was measured by dual-energy X-ray absorptiometry (DXA) using the Hologic QDR-1000 instrument (Waltham, MA). The validity and reliability of DXA measurements of body composition have been established (13, 14).

Blood sampling and analysis

Subjects reported to the laboratory between 0800 and 0900 after a 12-h fast. Subjects who were involved in physical training reported to the laboratory ≈24 h after their last physical training session. Blood for hemostatic measures was collected in citrate-containing tubes and immediately centrifuged (4500 × *g* for 10 min) at 4°C; plasma was stored at −70°C until analyzed. Blood samples for hemostatic measures (fibrinogen, PAI-1 antigen, and D-dimer) were analyzed at the Laboratory for Clinical Biochemistry Research, Department of Pathology, University of Vermont, Burlington. Fibrinogen, PAI-1 antigen, and D-dimer were chosen for study because they give a global view of coagulation and fibrinolysis (15). Fibrinogen was measured by an automated clot rate assay (16) using the ST4 instrument (Diagnostica Stago/American Bioproducts, Parsippany, NJ) with College of American Pathologists reference material. Proficiency was checked with the College of American Pathologists Coagulation Proficiency Testing Program. Both frozen and lyophilized controls were used. The typical CV for this assay is 2.9%. PAI-1 antigen was measured with an enzyme-linked immunosorbent assay (ELISA) originally developed by DeClerck et al (17). This method is sensitive to the free forms of PAI-1, both latent and active, but not to the complexed forms. The typical CV for this assay is 8.4%. D-Dimer was measured by using an ELISA method that uses 2 monoclonal antibodies directed against specific, nonoverlapping antigenic determinants that detect fragments of cross-linked fibrin. This assay does not detect fragments of non-cross-linked fibrin or fibrinogen (18). The typical CV for this assay is 7.0%. Reagents for the PAI-1 and D-dimer assays were generously provided by D Collen and P DeClerck in Leuven, Belgium.

TABLE 1
Baseline characteristics of subjects[1]

Variable	
Age (y)	9.5 ± 1.0
Height (cm)	140.4 ± 9.0
Weight (kg)	53.4 ± 13.6
BMI (kg/m^2)	26.8 ± 4.9
Ethnicity (black:white:Asian)	19:23:1

[1] $\bar{x}$ ± SD; *n* = 14 boys, 29 girls.

Physical training program

The physical training program was offered 5 d/wk, children were paid $1/session for each day of attendance and they were given prizes for satisfactory participation (ie, maintenance of heart rate >150 beats/min). Children were transported on school buses to the physical training center after school and home after training. Each 40-min physical training session was divided into two 20-min halves. During the first half, the children exercised on machines (treadmills, cycles, Nordic ski machines, minitrampolines, and rowing machines), typically spending 5 min on each of 4 machines. During the second half, children played group games designed to ensure that they were continuously active. In addition to minimizing any waiting to participate in the games, the children kept their heart rates elevated between activities by jumping on minitrampolines, bench-stepping, rope-jumping, or running and jumping in place while waiting their turn.

Each child wore a heart rate monitor (Polar Vantage, Port Washington, NY) during every session. After each session, the minute-by-minute values were downloaded to a computer and displayed to the child. Points were earned for maintaining the target heart rate and prizes were given after accumulation of a specified number of points (approximately every 2 wk).

To estimate the energy expenditure during the physical training sessions, each child underwent 2 multistage tests during the 4-mo physical training period in which oxygen consumption ($\dot{V}O_2$) and heart rate were measured. $\dot{V}O_2$ was measured with indirect calorimetry by using a FITCO (Farmingdale, NY) metabolic measurement cart and heart rate was measured with a Polar monitor. The test started at a power output of 30 W and was increased by 15 W every 2 min until a heart rate of ≈180 beats/min was reached. The regression of $\dot{V}O_2$ and $\dot{V}CO_2$ on heart rate was calculated for each child and the energy expenditure was estimated from the child's average heart rate during the physical training session.

Statistical analysis

The primary analysis in this study tested the effect of physical training and its cessation on hemostatic variables by using a group × time mixed-model analysis of variance (ANOVA) over the 3 time points (months 0, 4, and 8). Subject was considered a random factor, with group and time as fixed factors. This procedure allowed unequal sample sizes at different time points, so that the maximum number of subjects could be utilized in the analysis. Measurements that were not available at a given time point did not cause other observations for that subject to be excluded from the analysis because the missing observations were estimated from subject, time, and group totals by using a least-squares procedure. Least-squares means provide estimates of the expected values of the group means if the design was balanced. Thus, least-squares means were used in construction of the tables.

A second research question concerned the correlates of individual differences in the changes in hemostatic variables from

TABLE 2
Blood variables, fatness, and fitness changes over 8 mo in obese children[1]

Variable and group	Month 0	Month 4	Month 8
Fibrinogen (g/L)			
1	3.06 ± 0.07	2.92 ± 0.08	2.87 ± 0.10
2	3.17 ± 0.08	2.86 ± 0.08	2.90 ± 0.08
PAI-1 (μg/L)			
1	58.7 ± 6.1	47.0 ± 6.9	69.0 ± 8.0
2	48.8 ± 6.5	46.0 ± 6.5	58.0 ± 6.8
D-Dimer (μg/L)			
1	77.1 ± 6.1	58.9 ± 6.9	67.5 ± 8.0
2	89.3 ± 6.5	75.7 ± 6.5	53.7 ± 6.8[2]
Percentage body fat (%)			
1	42.6 ± 0.3	42.1 ± 0.3	43.0 ± 0.3
2	43.5 ± 0.3	44.2 ± 0.3	42.4 ± 0.3[3]

[1] $\bar{x}$ ± SE. Group 1 (n = 22) was involved in physical training in months 0–4 and group 2 (n = 21) was involved in physical training in months 4–8. PAI-1, plasminogen activator inhibitor 1.
[2,3] Group × time interaction effect: [2] $P = 0.08$, [3] $P < 0.01$.

before to after training. For this analysis, a difference score was obtained for each subject from just before to just after the physical training (ie, for subjects in group 1, the month 0 value was subtracted from the month 4 value, whereas for group 2 the month 4 value was subtracted from the month 8 value). The significance of the change from before to after physical training was assessed by a within-groups t test.

Pearson correlations were performed between changes in hemostatic measures and the independent variables. Stepwise regressions were done to evaluate the contribution of the independent variables to the change after physical training of each hemostatic variable. The independent variables were divided into 4 blocks: *1*) descriptive, *2*) hemostatic variables before physical training, *3*) body composition before physical training, and *4*) change in body composition from before to after physical training. For each hemostatic variable, a stepwise regression of the significant correlations ($P < 0.05$) was run separately for each block. The variables from each block that were retained were then entered together into a stepwise regression to obtain a final model. Because only one variable was retained in each of the final models, there was no opportunity to test for interactions. The cutoff P value for a variable to stay in the model was < 0.05. All statistical analyses were performed by using SAS version 6.12 (SAS Institute Inc, Cary, NC).

RESULTS

The mean (±SD) attendance was 78 ± 18% (ie, ≈4 d/wk) for the children who completed 4 mo of physical training. The heart rate and energy expenditure per physical training session were 157 ± 7 beats/min (≈78% of predicted maximal heart rate) and 967 ± 229 kJ (231 ± 55 kcal), respectively. These values indicate that subjects underwent rigorous physical training.

Values of the outcome variables (untransformed data) at each time point, and the P values for the group × time interactions, using analyses of log-transformed data for fibrinogen, PAI-1, and D-dimer, are shown in **Table 2**. No significant group × time interactions were found for the hemostatic variables. Thus, the main analysis did not provide evidence that the physical training or its cessation had a significant effect on the hemostatic measurements. Although the group × time interaction was not significant for D-dimer ($P = 0.08$), there was a decrease after physical training in both groups and an increase after no physical training in group 1. This pattern was similar to the significant pattern seen for percentage body fat; ie, values for both groups declined during the periods when they were engaged in physical training and those for group 1 increased after cessation of physical training.

TABLE 3
Changes from before to after physical training for cardiovascular disease risk factors and independent variables for groups 1 and 2 of obese children combined[1]

ΔFibrinogen (g/L)	−0.065 ± 0.381 (−1.35–0.77)
ΔPAI-1 (μg/L)	−1.9 ± 31.2 (−69.0–54.0)
ΔD-Dimer (μg/L)	−17.7 ± 26.2[2] (−77.3–60.4)
ΔFat mass (kg)	−0.3 ± 1.5 (−2.9–4.2)
ΔFat-free mass (kg)	1.6 ± 1.1[2] (−0.1–3.8)
ΔBMI (kg/m^2)	−0.1 ± 0.9 (−2.2–1.9)
ΔPercentage body fat (%)	−1.1 ± 1.9[2] (−4.6–2.5)

[1] $\bar{x}$ ± SD; range in parentheses. n = 38. PAI-1, plasminogen activator inhibitor 1.
[2] Significantly different from zero, $P < 0.01$.

The mean change scores from before physical training to after physical training for the independent and dependent variables are given in **Table 3**. This analysis was done to examine the determinants of individual differences in responsiveness to the physical training. Because the prephysical training value was subtracted from the postphysical training value, a negative score indicated a reduction in the variable. Of the hemostatic variables, only D-dimer decreased significantly from before to after physical training ($P < 0.05$). Of the total body-composition variables, fat mass and BMI did not change significantly. Fat-free mass increased significantly, as would be expected in growing children over a 4-mo period, and percentage body fat decreased significantly from before to after physical training ($P < 0.05$).

TABLE 4
Pearson correlation coefficients between changes from before to after physical training in hemostatic factors and potential determinants of change of obese children[1]

	Δ		
	Fibrinogen	PAI-1	D-Dimer
Age (y)	0.02	−0.20	−0.12
Sex (boy or girl)	0.03	0.05	0.10
Ethnicity (black or white)	−0.01	−0.05	−0.40[2]
Group (1 or 2)	0.23	0.33[2]	0.02
Before physical training			
Fibrinogen (g/L)	−0.61[2]	−0.09	−0.42[2]
PAI-1 (μg/L)	0.02	−0.40[2]	−0.14
D-Dimer (μg/L)	−0.33[2]	−0.08	−0.59[2]
Fat mass (kg)	−0.24	0.10	−0.32[2]
Fat-free mass (kg)	0.03	−0.24	−0.16
BMI (kg/m^2)	−0.21	0.15	−0.38[2]
Percentage body fat (%)	−0.39[2]	0.26	−0.38[2]
Change with physical training			
Fat mass (kg)	−0.28	0.09	−0.24
Fat-free mass (kg)	0.04	−0.06	−0.15
BMI (kg/m^2)	−0.17	−0.12	−0.16
Percentage body fat (%)	−0.25	0.10	−0.16

[1] Group 1 had physical training in months 0–4 and group 2 had physical training in months 4–8. n = 38. PAI-1, plasminogen activator inhibitor 1.
[2] $P < 0.05$.

TABLE 5
Final stepwise regression models[1]

Dependent variable	Independent variable	Partial R^2	Model R^2
ΔFibrinogen (g/L)	Fibrinogen before physical training	0.37 (−)[2]	0.37
ΔPAI-1 (μg/L)	PAI-1 before physical training	0.16 (−)	0.16
ΔD-Dimer (μg/L)	D-Dimer before physical training	0.35 (−)	0.35

[1] PAI-1, plasminogen activator inhibitor 1.
[2] Direction of change from before to after physical training (− or +) in parentheses.

Although 2 of the 3 hemostatic variables showed no significant changes for the subjects as a whole, there was a good deal of individual variation in the response to physical training. For example, although fibrinogen decreased by only 0.065 g/L (6.5 mg/dL) on average, one child had a decrease of 1.35 g/L (135 mg/dL), whereas another had an increase of 0.77 g/L (77 mg/dL).

The correlations between the changes in hemostatic risk factors and the independent variables are shown in **Table 4**. There were group differences in the response of PAI-1 to the physical training; values in group 1 decreased more from before physical training to after physical training than did those for group 2 ($P = 0.04$). Higher values before physical training of fibrinogen and D-dimer concentrations, and percentage body fat were associated with greater reductions in fibrinogen after physical training ($P < 0.05$). Higher concentrations of PAI-1 before physical training were associated with greater reductions in PAI-1 ($P < 0.05$). Higher concentrations before physical training of fibrinogen and D-dimer and values of fat mass, BMI, and percentage body fat were all associated with greater reductions in D-dimer ($P < 0.05$). Hence, those individuals with higher concentrations of hemostatic factors or greater adiposity showed more response to physical training. Blacks also had greater reductions in D-dimer concentrations than whites ($P < 0.05$). There were no significant associations between the changes in independent variables and the changes in hemostatic variables.

The final stepwise regression models predicting the hemostatic changes are given in **Table 5**. Children with higher fibrinogen concentrations before physical training had the greatest decreases in fibrinogen after physical training, accounting for 37% of the variance. Children who had higher PAI-1 concentrations before physical training showed greater decreases in PAI-1, accounting for 16% of the variance. When all the significant correlates were entered in a stepwise regression to predict D-dimer concentration, children with higher concentrations of D-dimer before physical training were those who showed greater decreases in D-dimer with physical training, accounting for 35% of the variance.

DISCUSSION

The primary analysis to test the effect of physical training and its cessation was the group × time ANOVA over the 3 time points. Because none of these interactions were significant for the hemostatic variables, this study did not provide evidence that physical training influenced the hemostatic measures. However, the interaction for D-dimer approached significance ($P = 0.08$) and the change from before to after physical training was significant ($P < 0.05$). Therefore, it may be appropriate to continue examining these variables in physical training programs that last longer than 4 mo and that produce greater reductions in percentage body fat. To our knowledge, there are no other studies in the literature documenting the effects of regular physical training on D-dimer concentrations in children.

Subjects in our study had baseline D-dimer concentrations several times greater than those reported for a similar age group of healthy children (20), perhaps because of their greater adiposity (11). Excess adiposity may cause an imbalance in the hemostatic system wherein more fibrin is produced and deposited, less is degraded, or a combination of both. Regular physical training that leads to decreases in adiposity could reduce D-dimer concentrations through a reduction in fibrin production or through increased fibrin breakdown. After 4 mo of no physical training, group 1 showed an increase in percentage body fat and D-dimer concentration, which further supports the possible role of exercise in influencing D-dimer concentrations. There were no significant changes in fibrinogen and PAI-1 concentrations after physical training. Some studies in adults have found similar results, whereas other studies have noted decreases in both variables (reviewed in 10).

There was a great deal of individual variation in the change in hemostatic variables, prompting us to explore determinants of change in these variables; to our knowledge, no other studies in the literature have looked at this issue in this way. We found that black children showed greater reductions in D-dimer concentration, possibly because of their higher baseline concentrations. Concentrations before physical training in our black subjects were 82% higher than those of our white subjects (89 compared with 49 μg/L). This is important because higher concentrations in adults are associated with increased risk for future myocardial infarction (9).

We found that those individuals with greater initial percentage body fat had greater reductions in fibrinogen and D-dimer concentrations from before to after physical training. Additionally, higher fat mass and BMI before physical training were associated with greater reductions in D-dimer concentrations. Thus, children with greater adiposity and concentrations of hemostatic species profited most from the physical training. When the final stepwise regression procedure was complete, baseline D-dimer concentration was the only variable in the model, explaining 35% of the variation in changes in D-dimer. It is possible that these results were due to statistical regression to the mean. However, the mean change from before to after physical training in D-dimer concentration (21%) was considerably greater than the error of the measurement (7%), and so argues against this. Studies of longer duration and studies that produce greater reductions in adiposity, perhaps by combinations of exercise and diet, are needed to provide more information about the extent to which reduction in adiposity may lead to favorable changes in hemostatic variables.

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