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Effect of Increased Exercise in School Children on Physical Fitness and Endothelial Progenitor Cells

A Prospective Randomized Trial

Claudia Walther, MD; Luise Gaede, BS; Volker Adams, PhD; Götz Gelbrich, MD, PhD; Alexander Leichtle, MD; Sandra Erbs, MD; Melanie Sonnabend, MS; Kati Fikenzer, MD; Antje Körner, MD; Wieland Kiess, MD; Mathias Bruegel, MD; Joachim Thiery, MD; Gerhard Schuler, MD

***Background*—**The aim of this prospective, randomized study was to examine whether additional school exercise lessons would result in improved peak oxygen uptake (primary end point) and body mass index–standard deviation score, motor and coordinative abilities, circulating progenitor cells, and high-density lipoprotein cholesterol (major secondary end points).

***Methods and Results*—**Seven sixth-grade classes (182 children, aged 11.1±0.7 years) were randomized to an intervention group (4 classes with 109 students) with daily school exercise lessons for 1 year and a control group (3 classes with 73 students) with regular school sports twice weekly. The significant effects of intervention estimated from ANCOVA adjusted for intraclass correlation were the following: increase of peak $\dot{V}O_2$ (3.7 mL/kg per minute; 95% confidence interval, 0.3 to 7.2) and increase of circulating progenitor cells evaluated by flow cytometry (97 cells per 1×10^6 leukocytes; 95% confidence interval, 13 to 181). No significant difference was seen for body mass index–standard deviation score (−0.08; 95% confidence interval, −0.28 to 0.13); however, there was a trend to reduction of the prevalence of overweight and obese children in the intervention group (from 12.8% to 7.3%). No treatment effect was seen for motor and coordinative abilities (4; 95% confidence interval, −1 to 8) and high-density lipoprotein cholesterol (0.03 mmol/L; 95% confidence interval, −0.08 to 0.14).

***Conclusions*—**Regular physical activity by means of daily school exercise lessons has a significant positive effect on physical fitness ($\dot{V}O_2$max). Furthermore, the number of circulating progenitor cells can be increased, and there is a positive trend in body mass index–standard deviation score reduction and motor ability improvement. Therefore, we conclude that primary prevention by means of increasing physical activity should start in childhood.

***Clinical Trial Registration*—**URL: http://www.clinicaltrials.gov. Identifier: NCT00176371.
(*Circulation*. 2009;120:2251-2259.)

Key Words: progenitor cells ■ exercise ■ obesity ■ pediatrics ■ prevention

Childhood obesity is the hallmark of an accelerating process that started in affluent Western societies decades ago. The dramatic increase in the prevalence of childhood overweight and obesity is related to comorbidities such as metabolic syndrome, diabetes mellitus, and other chronic diseases, including early atherosclerosis.[1–3]

Editorial see p 2168
Clinical Perspective on p 2259

The widespread increase in obesity rates has been too rapid to consider genetic factors as the primary cause, and therefore changes in young people's lifestyles, in particular an increase in sedentary behavior and a concomitant decline in physical activity,[4] have been the focus of most discussions.

Physical exercise has become the mainstay of primary and secondary prevention of cardiovascular diseases. Its preventive value has been proven over the last decades.[5,6] Unfortunately, in the majority of adult patients, persistent motivation and discipline to adhere to a regular training schedule are lacking. However, behavioral traits, which become quite rigid in adult life, may still be amenable to changes in childhood. It may therefore be postulated that an early adaptation to regular physical activity may result in a long-lasting positive effect on an active lifestyle.

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From the Department of Cardiology, Heart Center, University of Leipzig (C.W., L.G., V.A., S.E., M.S., K.F., G.S.); Coordination Center for Clinical Trials Leipzig (G.G.); University Hospital for Children and Adolescents, University of Leipzig (A.K., W.K.); and Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig (A.L., M.B., J.T.), Leipzig, Germany.

Correspondence to Claudia Walther, MD, Department of Cardiology, University of Leipzig, Heart Center, Strümpellstrasse 39, 4289 Leipzig, Germany. E-mail waltherc@medizin.uni-leipzig.de

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One of the markers associated with physical fitness and cardiovascular risk profile is circulating endothelial progenitor cells (CPCs).[7,8] CPCs originate from bone marrow stem cells and are involved in angiogenesis and key tissue repair mechanisms.[9,10] Impairment of the number and function of CPCs has been linked to increased atherosclerotic disease risk[11] and higher cardiovascular morbidity and mortality.[8] It has been demonstrated recently that adiposity impairs the number and function of CPCs in adults[12] and may therefore be contributing to adiposity-related cardiovascular risk. In addition, short- and long-term exercise regimens are known to have beneficial effects on the mobilization of CPCs in adults.[13–17]

The aim of this study was to investigate the effect of daily school exercise on physical fitness, motor skills, and body composition in school children through a randomized, prospective trial. Furthermore, we hypothesized that long-term physical exercise may result in persistent elevation of CPCs, reflecting improved tissue repair capabilities.

Methods

Study Design

A total of 188 children from 7 sixth-grade classes from 3 different schools were recruited. Because of removals and dropout of schools (3 students in the intervention group and 3 students in the control group), 182 students were included for baseline and follow-up analysis (Figure 1). After the rationale, study protocol, and potential side effects were explained, parents of all study participants gave informed consent. The investigational protocol was approved by the local ethics committee.

Study selection was based on the willingness of parents to allow their children to participate in the study protocol for at least 1 year. There were no exclusion criteria.

Randomization was performed by classes because of school curriculum requirements. Classes were randomly assigned to the intervention group (4 classes) or the control group (3 classes). According to German standards, 2 units (each 45 minutes) of physical education per week are mandatory in all schools. Intervention classes were assigned to 1 unit of physical exercise (45 minutes) with at least 15 minutes of endurance training per school day. The control classes continued to receive 2 units of exercise per week. In addition, lessons on healthy lifestyle were included in the regular schedule once monthly for all pupils.

In addition to the randomized classes, 2 additional sixth-grade classes from a school focusing on competitive sports and physical education were selected to serve as a reference group. The nonrandomized sport students (reference group) received 12 units (45 minutes per unit) of high-level endurance exercise training per week and frequently participated in competitive sporting events, thus representing a maximum of physical fitness attainable under reasonable conditions in school-age children.

Measurements were performed at baseline and after 1 year. These included assessment of body composition, blood pressure, heart rate, body coordination test, and treadmill exercise test with spirometry. Laboratory analyses such as lipid profile and the amount and the migratory function of CPCs were performed. Furthermore, the children had to complete the KINDer Lebensqualitätsfragebogen questionnaire[18] to evaluate health-related quality of life and eating behavior.

Anthropometry and Body Composition

Bioelectrical impedance for measurement of body composition was performed with the use of a soft tissue analysis/bioelectrical impedance analysis device (Akern/RJL, Florence, Italy), at a single frequency (50 Hz), with the participant lying relaxed on a couch with the arms and legs not in contact with other body parts.

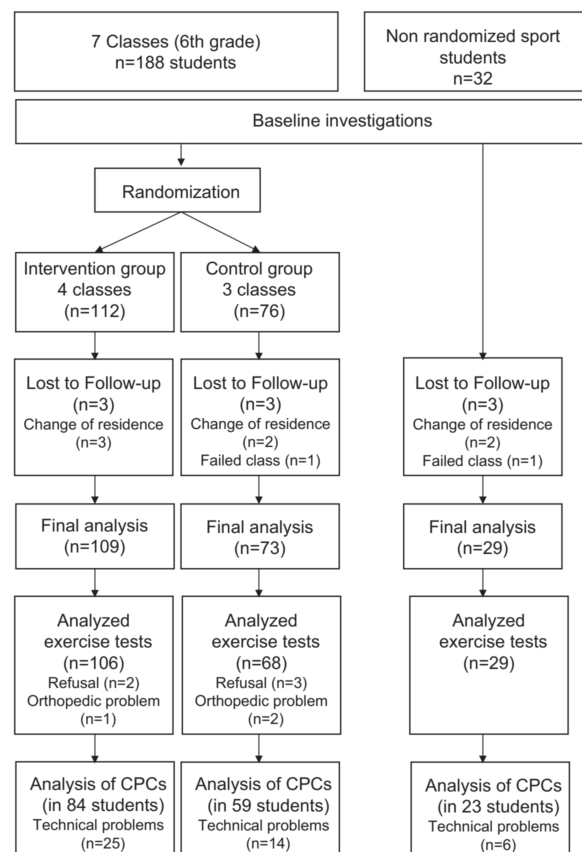

Figure 1. Flow diagram of dropouts during 1 year.

Sensing electrodes were placed over the right wrist and ankle; current electrodes were placed over the metacarpals and metatarsals. Weighing was performed with the children barefoot, wearing shorts and T-shirt on a digital scale (Tanita HD-317, Tanita Corporation, Tokyo, Japan) with a range of 0 to 150 kg and accuracy of 100 g. Height was measured with the children standing erect and barefoot against a flat vertical surface. Measurements were taken with a stadiometer (Sanny Kirchner & Wilhelm Medizintechnik, Germany) fixed to the wall. Capacity was 2 m, and precision was 0.1 cm.

Body mass index (BMI) and BMI–standard deviation score (BMI-SDS) were calculated. Children were defined as being overweight or obese according to the BMI-to-age ratio, with the use of limits proposed for German school children by the Arbeitsgemeinschaft Adipositas im Kindes- und Jugendalter.[19,20] Children over the 90th and 97th percentiles for BMI/age were considered overweight and obese, respectively.

Treadmill Exercise Test With Spirometry

Before they were tested, children were familiarized with the treadmill exercise test. All participants underwent a graded treadmill test (Woodway USA Inc, Waukesha, Wis) with spirometry until exhaustion, according to a modified Bruce protocol for children starting at 1.7 mph and 0 degrees.[21] The protocol continued until 1 of the end points (changes in ECG, hypotension or hypertension, fatigue, dyspnea, or arrhythmias) was reached. The recovery period lasted 5 minutes or until the heart rate and blood pressure normalized, whichever was longer. The exercise test was started when oxygen consumption ($\dot{V}O_2$) at rest showed stable levels. At the end of every exercise stage, children had to indicate their personal perception of exhaustion according to the Borg rating. In addition, blood pressure, heart rate, and $\dot{V}O_2$max obtained with the use of a portable spirometry system (K4b2, Cosmed Srl, Italy) with a dead space of 70 mL were recorded after each stage.

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Body Coordination Test for Children

The Body Coordination Test for Children is a reliable test battery for body coordination and motor skills in children aged 5 to 14 years.[22] The test contains 4 subtests of specific motor functions: balancing backward, jumping over a certain height, jumping sideways, and shifting platforms sideways. Every subtest is demonstrated and explained until the proband has a complete understanding of the task. For each test, the children had 3 attempts, and the results were added. Each score achieved in the subtests was adjusted for age and sex, resulting in the motor quotient, which indicates the child's overall motor ability. A motor quotient between 85 and 115 is considered normal.

Laboratory Testing

Peripheral blood samples were obtained before the specific tests were performed in all pupils. Analyses of total cholesterol, low-density lipoprotein, high-density lipoprotein cholesterol, and triglycerides were performed with an enzymatic colorimetric test (Roche Diagnostic, Hoffmann–La Roche, Ltd, Basel, Switzerland). Technicians were blinded for group assignments.

Measurement of CPCs

For flow cytometry, a volume of 200 μL peripheral blood was incubated for 20 minutes with different combinations of the following antibodies: PE-conjugated mouse anti-human VEGFR2 (also known as KDR; R&D Systems), FITC-conjugated mouse anti-human CD34 (Miltenyi), PerCP-conjugated mouse anti-human CD3, APC-conjugated mouse anti-human CD45 (BD Pharmingen), and PE-conjugated mouse anti-human CD133 (Miltenyi). The erythrocytes were lysed after incubation, and the remaining cells were washed with phospate-buffered saline and fixed in 2% paraformaldehyde before analysis with the use of a FACS-Calibur (Becton-Dickinson). CPCs were defined as $KDR^+/CD34^+$ cells or $CD45^{low}/KDR^+/CD34^+$ cells as described recently.[16,17] Technicians evaluating the number of CPCs were blinded for group assignments.

Measurement of Migratory Capacity of Endothelial Progenitor Cells

Mononuclear cells were isolated from venous blood by density gradient centrifugation (Histopaque 1077, Sigma, Deisenhofen, Germany). After the cells were washed several times with phosphate-buffered saline, 1×10^7 cells were plated on gelatin-coated cell culture dishes (6-well plates; TPP, Berlin, Germany) and cultured in EBM-2 (Cambrex, Verviers, Belgium) supplemented with 10% fetal calf serum (Biochrom KG, Berlin, Germany), 100 U penicillin, 100 μg/mL streptomycin, and the following growth factors: basic fibroblast growth factor (4 ng/mL), vascular endothelial growth factor (2 ng/mL), epidermal growth factor (10 ng/mL), insulin-like growth factor-1 (5 ng/mL), hydrocortisone (200 ng/mL), and ascorbic acid (75 ng/mL). After 7 days in culture, the cells were detached with the use of trypsin/EDTA, and the migratory capacity of the cells toward stromal cell–derived factor-1 (100 ng/mL) was evaluated with the use of a modified Boyden chamber, as described recently.[16] Technicians evaluating the migratory function of CPCs were blinded for group assignments.

Statistical Analysis

Hypotheses

The primary end point of the study was the change in $\dot{V}O_2$max after 1 school year. On the basis of the results of the pilot study,[23] we hypothesized achievement of an increase in $\dot{V}O_2$max of 4 mL/min per kilogram by the intervention group compared with the control group. Further hypotheses on the major secondary end points stated the following effects of the intervention: decrease of BMI-SDS, increase of motor quotient score, increase in percentage of $CD45^{low}/CD34^+/KDR^+$ cells, and increase in high-density lipoprotein serum cholesterol.

Table 1. Baseline Characteristics of Students

	Intervention Group	Control Group
n	109	73
Boys/girls, %	53/47	58/42
Age, y	11.1±0.7	11.1±0.7
Height, cm	150.9±7.2	150.7±8.1
Weight, kg	41.3±8.4	41.5±8.7
BMI, kg/m^2	18.0±2.6	18.2±2.8
BMI percentile	50.5±28.9	52.5±28.8
Heart rate at rest, bpm	101±13	103±12

Sample Size

With a 4:3 ratio for randomization (4 school classes with intervention and 3 control classes), an estimated SD of $\dot{V}O_2$max within groups of 7 mL/min per kilogram, a type I error of 0.05, and a statistical power of 0.85, the required sample size would be 115 (66 intervention and 49 control subjects) for independent subjects. With the assumption of a SD of 1 mL/min per kilogram for the variability between classes, the anticipated intraclass correlation coefficient is 0.02. The sample size adjusted for the cluster effect[24] is then 168 (ie, 7 school classes with 24 subjects per class should be evaluable). Allowing for 2 dropouts per class, 182 subjects should be enrolled. This target was achieved as eventually 188 subjects were included.

Methods of Analysis

Quantitative variables are expressed as mean±SD throughout the article unless stated otherwise. Comparisons between groups with respect to all quantitative end points were performed by ANCOVA with the follow-up measurement as dependent variable, treatment group as factor, and the baseline measurement as covariate. Estimates for the treatment effect are presented as mean values with 95% confidence intervals. Standard errors of estimates and hence confidence intervals and *P* values were adjusted for intraclass correlation following the methods described by Ukoumunne and coworkers.[24] Further auxiliary analyses should be seen in a descriptive context, even if formal procedures of inference statistics were used. A baseline multivariable regression analysis was performed to explore relationships between CPCs and risk factors, anthropometric measures, and physical capacity. The 2-tailed Student unpaired *t* test was used for comparisons between the randomized sample and a nonrandomized reference group of sports students.

SPSS 15 (SPSS Inc, Chicago, Ill) was used for all analyses. A P value of <0.05 was considered statistically significant for the primary outcome.

Results

Intervention Versus Control Group

Demographics

A total of 182 students from 7 classes (89 boys, 93 girls; mean age, 11.1±0.7 years) of 3 different schools took part in the study, with 109 and 73 children in the intervention group and control group, respectively. Baseline characteristics are shown in Table 1. At baseline, 12.8% of intervention group students and 10.9% of control group students were obese or overweight (≥90th percentile). After 1 year, remission from overweight to normal weight in 4 students of the intervention group and an incidence of overweight/obesity in 2 normal-weight children of the control group were observed (Figure 2). After adjustment for intraclass correlation, no significant effect on BMI-SDS was detected (Table 2). With respect to nutritional behavior, no differences could be detected between the groups at baseline and after 1 year.

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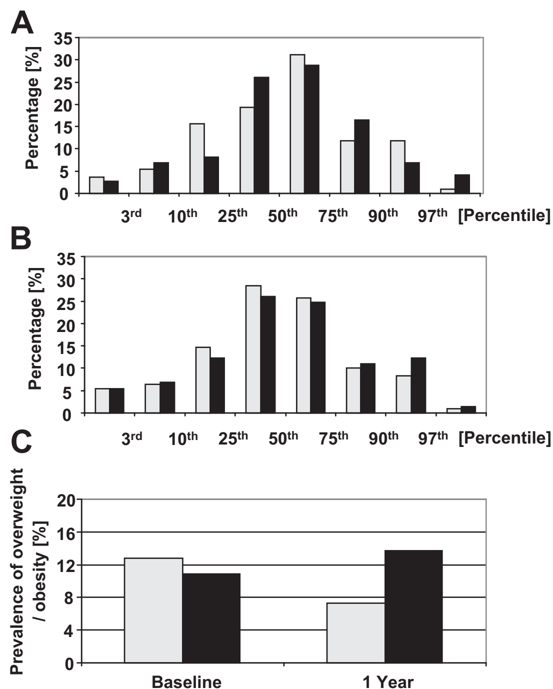

Figure 2. Percentage of children in different BMI percentiles at baseline (A) and after 1 year (B) of the study and the prevalence of children with a BMI ≥90th percentile at baseline and after 1 year (C). Gray bars indicate intervention group; black bars, control group.

Exercise Capacity and Motor Abilities

Treadmill exercise testing with spirometry was successfully performed in 106 children of intervention group and 68 children of control group (Figure 1). Exercise and motor variables are depicted in Table 2. Maximal oxygen consumption ($\dot{V}O_2$max) was comparable in both groups at baseline. After 1 year, a significant treatment effect was seen in the comparison between groups (3.7 mL/kg per minute; 95% confidence interval, 0.3 to 7.2; $P=0.032$). This result was accompanied by significant gains due to the intervention in maximum workload and in oxygen uptake at maximum exertion (Table 2 and Figure 3).

Motor abilities improved in both groups and were stronger in the intervention group, but formal significance of the treatment effect was not achieved after adjustment for intraclass correlation. Six percent of the intervention group students and 7% of the control group students were considered to have disordered motor functions at baseline. This proportion decreased to 0% in the intervention group and to 6% in the control group after 1 year.

Laboratory Analysis

Lipid Profile

The lipid profiles of the different groups are shown in Table 2. No essential differences between groups were observed.

Endothelial Progenitor Cells

In the majority of students, fluorescence-activated cell sorter scan quality was high enough to analyze the amount of CPCs (CD34 or $CD34^+/KDR^+$ or $CD45^{low}/CD34^+/KDR^+$). At baseline, no significant difference of circulating $CD34^+$, $CD34^+/KDR^+$, or $CD45^{low}/CD34^+/KDR^+$ cells could be detected between the control group and the intervention group (Table 2).

After adjustment for intraclass correlation, an increase in $CD45^{low}/CD34^+/KDR^+$ cells was observed in the intervention group after 1 year (97 cells per 1×10^6 leukocytes; 95% confidence interval, 13 to 181) (Table 2 and Figure 4). No significant treatment effect could be detected with respect to $CD34^+$ and $CD34^+/KDR^+$ cells and migratory capacity (Table 2).

In addition, a baseline multivariable analysis exploring relationships between the CPC concentration (as dependent variable) and variables such as fat-free mass, total cholesterol, resting systolic blood pressure, and high-density lipoprotein cholesterol was performed. Regression analysis revealed an R^2 of 0.032 for the model including the 4 selected parameters, with an ANOVA significance of 0.222.

Group of Nonrandomized Sport Students

A total of 29 children (12 girls and 17 boys; mean age, 11.4±0.5 years) from a specific school focusing on competitive sports and physical education were examined as reference group. Sport students had larger body heights (154.5±7.4 cm), lower BMI (17.1±1.6 kg/m^2), lower BMI-SDS (−0.3±0.65), higher percentages of fat-free mass (87.3±6.8%), and lower heart rate at rest (96±10 bpm) than control group/intervention group students. None of the students in the reference group was overweight (BMI >90th percentile) at baseline or after 1 year.

$\dot{V}O_2$max was 47.8±7.7 mL/kg per minute in the reference group compared with 37.9±5.7 mL/kg per minute in the intervention group ($P<0.001$, adjusted for clustering) and 38.8±4.8 mL/kg per minute in the control group ($P<0.001$, adjusted for clustering) at baseline. At follow-up, $\dot{V}O_2$max was 60.6±6.3 mL/kg per minute in the reference group compared with 48.8±6.8 mL/kg per minute in the intervention group ($P<0.001$) and 45.6±8.9 mL/kg per minute in the control group ($P<0.001$). The mean values of the intervention group and the control group were therefore 79.3% and 81.2% of the reference group at baseline and 80.5% and 75.2% of the reference group at follow-up, respectively.

$CD45^{low}/CD34^+/KDR^+$ cell counts per milliliter blood were 524±134 in the reference group compared with 305±132 in the intervention group ($P<0.001$) and 308±103 in the control group ($P<0.001$) at baseline, and they were 497±155 in the reference group compared with 359±173 in the intervention group ($P=0.006$) and 263±143 in the control group ($P<0.001$) at follow-up. The mean values of the intervention and control groups were 58.2% and 58.8% of the reference group at baseline and 72.2% and 52.9% of the reference group at follow-up, respectively.

Discussion

The prevalent inactive lifestyle of children in developed countries is associated with a multitude of harmful trends including obesity, early diabetes mellitus, and hypertension. The central message emerging from this study is that some of these trends may be reversed and possibly corrected by increasing the level of physical activity during school age:

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Table 2. ANCOVA Analysis of Primary and Secondary Outcomes at Baseline and After 1 Year

	Treatment Group		Difference Intervention Group−Control Group*	
Variable	Intervention	Control	Estimate (95% Confidence Interval)	*P*
Primary end point				
$\dot{V}O_2$max, mL/min per kilogram	n=106	n=68		
Baseline	37.9±5.7	38.8±4.8		
Follow-up	48.8±6.8	45.6±8.9	3.7 (0.3 to 7.2)	0.032
Major secondary end points				
BMI-SDS	n=109	n=73		
Baseline	−0.01±1.00	0.07±1.00		
Follow-up	−0.24±1.04	−0.10±1.05	−0.08 (−0.28 to 0.13)	0.472
Motor quotient score	n=107	n=69		
Baseline	109±13	108±13		
Follow-up	116±10	112±15	4 (−1 to 8)	0.108
$CD45^{low}/CD34^{+}/KDR^{+}$, $1/10^6$ leukocytes	n=84	n=59		
Baseline	305±132	308±103		
Follow-up	359±173	263±143	97 (13 to 181)	0.023
HDL cholesterol, mmol/L	n=105	n=57		
Baseline	1.38±0.31	1.47±0.42		
Follow-up	1.42±0.33	1.47±0.37	0.03 (−0.08 to 0.14)	0.623
Other measures				
Spiroergometry				
$\dot{V}O_2$ per beat, mL/beat per kilogram	n=106	n=68		
Baseline	0.191±0.029	0.192±0.026		
Follow-up	0.238±0.034	0.221±0.046	0.017 (0 to 0.034)	0.049
Physical work capacity, W	n=106	n=68		
Baseline	133±29	134±28		
Follow-up	154±37	146±32	9 (2 to 17)	0.019
Maximal heart rate, bpm	n=106	n=68		
Baseline	198±9	202±8		
Follow-up	206±11	207±9	2 (−1 to 4)	0.149
Maximal systolic RR, mm Hg	n=106	n=68		
Baseline	129±12	128±12		
Follow-up	138±12	139±12	−2 (−5 to 1)	0.232
Maximal diastolic RR, mm Hg	n=106	n=68		
Baseline	63±7	65±7		
Follow-up	54±8	54±8	0 (−3 to 3)	0.937
Body composition				
Fat-free mass, %	n=109	n=73		
Baseline	78.7±8.8	78.5±10.1		
Follow-up	78.3±9.6	76.5±9.6	1.7 (−3.0 to 6.4)	0.483
TBWC, %	n=109	n=73		
Baseline	58.8±7.7	58.5±7.9		
Follow-up	58.3±7.5	57.2±7.3	0.9 (−2.9 to 4.6)	0.653
Laboratory				
$CD34^{+}/KDR^{+}$, 1/mL blood	n=99	n=57		
Baseline	224±191	312±204		
Follow-up	256±235	273±243	17 (−111 to 146)	0.791
$CD34^{+}$, 1/mL blood	n=101	n=61		
Baseline	1269±1297	1485±867		
Follow-up	1679±1214	1740±1397	−52 (−802 to 697)	0.891

(*Continued*)

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Table 2. Continued

Variable	Treatment Group		Difference Intervention Group−Control Group*	
	Intervention	Control	Estimate (95% Confidence Interval)	*P*
Migratory capacity, cells/10^3 seeded cells	n=82	n=63		
Baseline	59.3±46.2	57.0±62.3		
Follow-up	58.1±64.4	62.9±50.9	−0.46 (−3.60 to 2.68)	0.774
LDL cholesterol, mmol/L	n=105	n=57		
Baseline	2.18±0.54	2.18±0.57		
Follow-up	2.17±0.50	2.14±0.65	0.04 (−0.14 to 0.21)	0.668
Total cholesterol, mmol/L	n=105	n=56		
Baseline	4.20±0.63	4.26±0.70		
Follow-up	4.19±0.60	4.12±0.66	0.11 (−0.13 to 0.35)	0.370
Triglycerides, mmol/L	n=105	n=56		
Baseline	1.10±0.46	1.10±0.51		
Follow-up	1.04±0.49	1.11±0.52	−0.08 (−0.29 to 0.14)	0.500

Data are mean±SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; TBWC, total body water content.
*Estimates from ANCOVA. Confidence intervals and *P* values are adjusted for intraclass correlation.

Students were physically fitter in the intervention group than their counterparts in the control group, CPCs were elevated, and a trend was seen for a reduction in BMI-SDS, which was an improvement of motor abnormalities and a lowering of the prevalence of overweight/obese children. Such findings were particularly notable given that the study participants were relatively healthy compared with published demographic data.[25] The prevalence of overweight/obese children was "only" 12.1% of the entire group before randomization. However, motor deficits were obvious in 6.0% of the students.

CPCs have a variety of positive effects on cardiovascular metabolism and function,[10] and improvements in CPC counts and mobility have been linked to increased exercise.[13–17] Students who attended daily exercise sessions showed a significant increase in CPC counts compared with the control group in the present study, although the levels were not as high as those observed in students performing regular exercise on a much higher level (reference group).

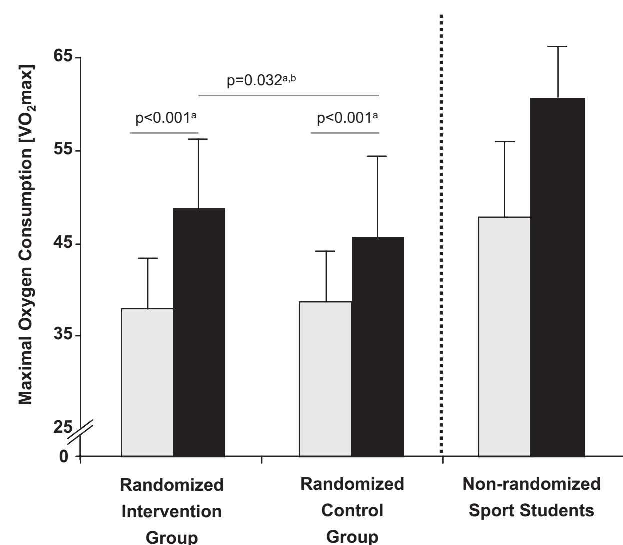

Figure 3. Maximal oxygen consumption of the intervention and control groups and nonrandomized sport students at baseline and after 1 year. Gray bars indicate baseline; black bars, 1 year. [a]Adjusted for clustering; [b]adjusted for baseline values.

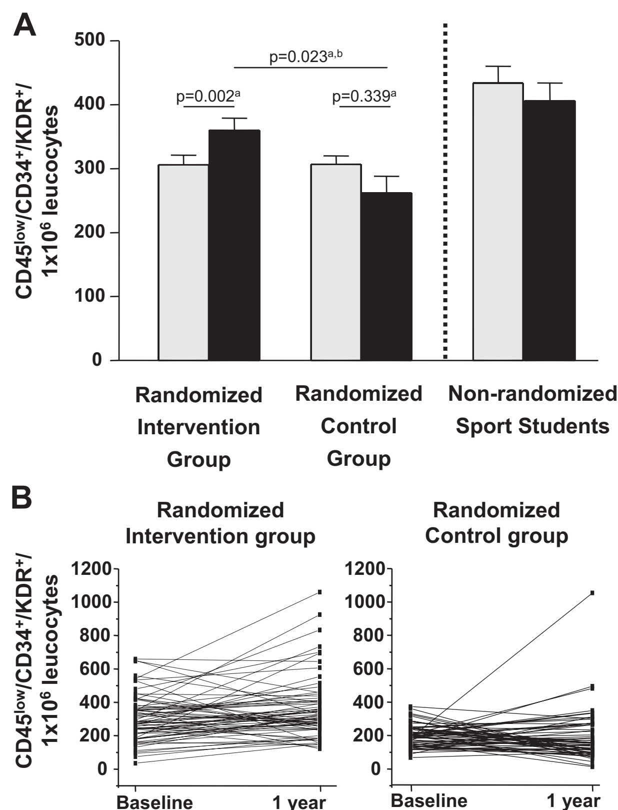

Figure 4. A, Amount of circulating endothelial progenitor cells defined as $CD45^{low}/CD34^+/KDR^+$ at baseline and after 1 year in the intervention and control groups and nonrandomized sport students. B, Individual data of $CD45^{low}/CD34^+/KDR^+$ at baseline and after 1 year in the intervention and control groups. Gray bars indicate baseline; black bars, 1 year. Data are presented as mean±SEM. [a]Adjusted for clustering; [b]adjusted for baseline values.

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Surprisingly, migratory capacity, as a measure of CPC function, also remained significantly below the levels observed in reference group students. One possible explanation is the difference in physical activity levels, which was less than half in intervention group compared with reference group students. It may be surmised that significantly higher intensities of exercise are required to improve CPC function than to increase their absolute number.

During the past several years, >100 intervention programs for preventing obesity in childhood have been initiated in various countries.[26–28] These programs differ with respect to duration of intervention, ranging from weeks to years, as well as varying intervention strategies such as diet, exercise, lifestyle, and social support. Methodological problems such as the nonrandomized design of such studies and the lack of objective measurements to assess physical fitness have led to inconclusive results. An insufficient amount of school-based physical activity was observed in the majority of these studies. Despite such limitations, it can be surmised that an increase in physical activity constitutes an important intervention to prevent or treat childhood obesity.[29–32]

The present study on exercise training in school children differs from existing studies in the literature in several important aspects: It is prospectively randomized, exercise sessions were part of the regular school curriculum, and complete classes were analyzed. As stated by world experts during the first conference of the International Association for the Study of Obesity, school and kindergarten are critical settings in which exercise interventional programs can best be implemented.[30] We decided to perform a school-based exercise study to minimize the effects of parents‘ lifestyle and/or attitudes toward physical activity in a setting that was irrespective of socioeconomic status. The amount of exercise chosen in the present study is based on the recommendations of current guidelines for physical activity in children.[33] Our results show that such an exercise program can be easily integrated into school curricula, with subsequent positive effects on exercise capacity and the amount of circulating CPCs in participants. Compared with interventional programs with dietary restrictions and lifestyle modifications (eg, prohibiting television watching), increasing physical activity is less prohibitive and thus may be perceived as positive rather than negative. This may have resulted in the high compliance rate of 100% in the present study.

An important goal of this study was to analyze a surrogate parameter for potential future cardiovascular risk because an increase in cardiac events cannot be expected at school age. CPCs were shown to be one of the most important factors in predicting cardiovascular morbidity and mortality, at least in patients with coronary artery disease.[8] Physical exercise and ischemia seem to facilitate the release of CPCs from the bone marrow, leading to an increased detectable number in the peripheral blood.[13–17] CPCs may be involved in angiogenesis and repair of endothelial integrity in areas that have sustained damage.[10] CPC efficacy is determined not only by their concentration in the peripheral circulation but also by their migratory capacity in reaching areas in need of repair. In the present study, the highest concentrations of CPCs were observed in reference group students, with CPC concentrations that were 30% higher than in the other groups at baseline. During the course of 1 year, CPCs increased significantly in the intervention group, reaching levels comparable to those of the reference group.

The baseline concentration of CPCs showed a large variability, probably due to the population-based design without a preselection of the children. However, the CPC concentration (mean, 500 $CD34^+/KDR^+$ cells per milliliter blood in all students) in the present study was ≈5 times higher than the amounts observed in elderly patients with cardiovascular disease.[13] Our results are in agreement with the observation of other authors who described an inverse relationship between the amount of CPCs and increasing age and cardiovascular risk factors.[11,13] The physiological role of CPCs in healthy children, however, is speculative. We hypothesize that the increased amount of CPCs observed in reference group and intervention group children is an expression of the long-term adaption to the increased exercise, possibly related to new vessel formation in the working muscle.

Contrary to what we expected, no alteration in CPC function, expressed by migratory capacity, was evident in intervention group children after 1 year compared with control children. However, CPCs isolated from children performing exercise training at a high level (reference group) exhibited a significantly better migratory capacity than CPCs of children from the other 2 groups. On the basis of these data, we may conclude that exercise training of 1 hour per day is sufficient to increase the amount of CPCs but not their function. Such a conclusion is supported by the results from the European Youth Heart Study,[34] in which the authors ascertained that 1 hour of daily physical activity, as recommended by current guidelines,[33] is probably an underestimation of the amount of physical activity necessary to prevent cardiovascular risk.

Study Limitations

The first limitation of the study is that it was cluster randomized with a small number of clusters. However, when we accounted for group assignment, there was still a significant effect in the intervention group relative to the primary outcome of $\dot{V}O_2$max.

Another limitation of the study is that the participating children were relatively healthy, with a small percentage of overweight and obese children (13%), compared with the prevalence of up to 25% as reported worldwide.[35] Therefore, the data obtained in this study may not be transferable to the whole population.

In regard to CPCs, a clear and standardized definition with respect to cell surface markers is not yet available. Therefore, several subpopulations of CPCs can be identified. This becomes obvious also in the present study, in which a significant change was only observed for $CD45^{low}/CD34^+/KDR^+$ cells but not for $CD34^+/KDR^+$ cells. In addition, the clinical relevance of each subpopulation is far from being clear.

Another limitation with the functional analysis of CPCs is that only their migratory capacity toward stromal cell–derived factor-1 was analyzed, which does not represent the

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entire functional repertoire (tubule formation, colony formation, and nitric oxide generation).

Summary

In summary, this is the first prospectively randomized study demonstrating that regular exercise training implemented into a daily school curriculum improves cardiovascular fitness levels, increases circulating progenitor cells in school children, and shows a trend toward the improvement in motor abilities and a reduction in BMI-SDS. These findings suggest that school curricula may be an effective vehicle for increasing physical activity and improving cardiovascular health for children. Longer-term observations (>5 years) will be required to determine whether our school-based exercise program is capable of leading to improved attitudes toward physical activities, improved exercise, and decreased cardiovascular risk in adulthood.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Childhood obesity is the result of a long-lasting imbalance between energy intake and energy expenditure. The decline of physical activity over the last decades is thought to be one of the main risk factors for the development of overweight and obesity and its comorbidities. Therefore, intervention strategies to treat and prevent childhood obesity are necessary. Physical exercise is one of the cornerstones of obesity prevention, and its preventive value has been proven over the last decades. An attractive setting to start and implement such an exercise program is in school, which is independent of environmental and parental influences. The aim of this prospective randomized trial was to compare sixth-grade students with daily school exercise lessons with a control group with 2 exercise lessons per week with respect to physical fitness, motor skills, body composition, and circulating endothelial progenitor cells as a surrogate parameter for cardiovascular risk/function and tissue repair capabilities. The central message emerging from this study is that some of the harmful trends associated with childhood obesity and sedentary behavior may be reversed by increasing the level of physical activity. In addition, daily exercise leads to an increase of circulating endothelial progenitor cells without improvement of their migratory function. Furthermore, the results demonstrate that implementation of daily exercise lessons into the school curriculum is feasible, well tolerated, and accepted.

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