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Research article

Improvements in Physical Fitness are Associated with Favorable Changes in Blood Lipid Concentrations in Children

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Abstract
Although accumulating evidence suggests the benefits of cardiorespiratory fitness and muscular fitness, little knowledge exists on how other physical fitness (PF) components are associated with cardiovascular disease (CVD) risk markers in children. Additionally, much of the relevant evidence is from longitudinal studies with CVD risk markers at a single time point (i.e., baseline) rather than changes in PF. The purpose of the present study was to examine whether initial 1-year changes in different performance measures of PF (i.e., endurance performance, muscular strength/endurance, flexibility, agility, and speed) can predict the subsequent changes (2-year change) in blood lipid concentrations in children. This 2-year longitudinal study included a total of 251 Japanese children (mean age 9.2 ± 0.4). PF tests were performed to comprehensively evaluate the participant's fitness levels (handgrip strength [upper body muscular strength], bent-leg sit-ups [muscular endurance], sit-and-reach [flexibility], side-step [agility], 20-meter shuttle run [endurance performance], 50-meter sprint [speed], standing long jump [lower body muscular strength], and softball throw [explosive arm strength and throwing ability]). Fasting lipid profile was assayed for triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and non-HDL-C concentration. Multilevel linear regressions were used to examine the associations between the preceding changes (over 1-year) in PF and subsequent changes (over 2-years) in blood lipid concentrations. We also examined the simultaneous associations between 2-year changes in PF and 2-year changes in blood lipid concentrations. For boys, preceding improvement in handgrip strength was negatively associated with TG concentration ($\beta = -0.260$, $p = 0.030$); improvements in bent-leg sit-ups were negatively associated with clustered lipid scores ($\beta = -0.301$, $p = 0.038$) and non-HDL-C ($\beta = -0.310$, $p = 0.044$); and improvements in 50m sprinting were associated with subsequent changes in non-HDL-C ($\beta = 0.348$, $p = 0.006$) and LDL-C ($\beta = 0.408$, $p = 0.001$). For girls, improvements in handgrip strength was negatively associated with TG concentration ($\beta = -0.306$, $p = 0.017$); and improvements in standing long jump were negatively associated with non-HDL-C ($\beta = -0.269$, $p = 0.021$) and LDL-C ($\beta = -0.275$, $p = 0.019$). For boys and girls, there were no significant simultaneous associations between 2-year changes in PF and 2-year changes in blood lipid concentrations. In conclusion, preceding change in physical fitness in relation to change in blood lipid concentration likely reflect a physiological adaptation to growth and maturation since these associations diminished in the subsequent year.

Key words: Youth, health, prospective study, physical strength.

Introduction

Physical fitness (PF), defined as an ability to perform daily activities consisting of morphological, muscular, motor, cardiorespiratory, and metabolic components (Malina and Katzmarzyk, 2006), is known as one of the strongest predictors of future cardiovascular disease (CVD) risk (Kodama et al., 2009; Ortega et al., 2008b). In research on young populations, much attention has been given to the beneficial effects of two specific PF components: cardiorespiratory fitness and muscular fitness. A recent systematic review and meta-analysis of 55 studies, which collectively followed up 37,563 youths (aged 3–18 at baseline) for a mean of 8.6 years, indicated that there were weak–moderate associations of cardiorespiratory fitness with waist circumference, skinfold thickness, obesity, blood lipids, insulin resistance and cardiometabolic risk at later in life (García-Hermoso et al., 2020). Additionally, a systematic review and meta-analysis of 30 studies with 21,686 youths (aged 3–18 at baseline) indicated that there were moderate–large associations of muscular fitness with skinfold thickness, insulin resistance, blood lipids, CVD risk markers, and bone mineral density at follow-up (García-Hermoso et al., 2019). Unlike the robust evidence regarding the benefits of cardiorespiratory fitness and muscular fitness, little knowledge exists on how other PF components (e.g., muscular endurance, agility, flexibility, and speed) are prospectively associated with CVD risk markers, including serum lipid levels in children (Roldão da Silva et al., 2020; Zaqout et al., 2016). Available evidence from longitudinal studies indicates that associations of different performance measures of PF with CVD risk markers are different according to PF tests as well as the study population. For example, a 2-year longitudinal study with 1,635 European children aged 6–11 showed that higher performance of some PF tests (i.e., cardiorespiratory fitness, lower body muscular strength, speed, and balance) was inversely associated with the follow-up CVD risk markers (i.e., waist circumference, blood pressure, blood lipids, and insulin resistance) while it was not for the other PF tests (i.e., upper body muscular strength and flexibility) (Zaqout et al., 2016). However, a 3-year longitudinal study with 93 Brazilian adolescents (13.8 ± 0.8 years) showed that there was no longitudinal association between PF tests (i.e., cardiorespiratory fitness, muscular fitness, flexibility) and the follow-up CVD risk markers (i.e., waist circumference, blood pressure, blood lipid and fasting blood glucose).

Prospective studies with children in this research area have typically examined associations of PF at baseline (i.e., a single point in time) with CVD risk markers at follow-up (Castro-Piñero et al., 2019; Fraser et al., 2016; Grøntved et al., 2015; Savva et al., 2014; Schmidt et al.,

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2016; Sun et al., 2014), while only limited evidence is available for the associations between change in PF (evaluated by cardiorespiratory fitness and muscular fitness) and simultaneous changes in CVD risk markers in children (Agostinis-Sobrinho et al., 2018a, 2018b). Furthermore, it is unknown how preceding changes in PF might predict subsequent changes in CVD risk markers. In adults, a longitudinal study with 3,261 Japanese adults (aged 25–74) demonstrated that initial 5-year changes in moderate-to-vigorous physical activity were associated with subsequent changes (12 years) in waist circumference (Shibata et al., 2016). Revealing chronological sequences between PF and CVD markers can provide us with more robust evidence on the potential cause and effect relationship between the two outcomes. However, no study has been conducted to examine the chronological sequence between PF and CVD markers in children. Therefore, the purpose of the present study was to examine whether initial 1-year changes in PF (endurance performance, muscular strength/endurance, flexibility, agility, and speed) would predict subsequent changes (2-year change) in blood lipid concentration in Japanese children. The present study also examined the simultaneous associations between 2-year changes in PF and 2-year changes in blood lipid concentrations.

Methods

Participants

The present study recruited children from three public primary schools in Saku-city, Nagano, Japan. All children in grade 4 (aged 9–10) attending the participating schools were invited to participate in the study. Baseline assessments were conducted when the participants were in 4th grade, and the follow-up assessments were conducted when the participants were in 6th grade (2-year follow-up). Figure 1 describes a sampling flow in the present study. We collected data from three cohorts: a 2015 cohort [investigation period: AY2015–2017], a 2016 cohort [investigation period: AY 2016–2018], and a 2017 cohort [investigation period: AY 2017–2019]. The data from the three cohorts were then combined for analysis. Fasting blood tests were conducted at baseline and two years later (two time points). PF tests were conducted at three time points: at baseline, one year later (1-year follow-up), and two years later (2-year follow-up). Fasting blood tests and PF tests were conducted in all years at the participating schools. All children and parents/guardians provided written, informed consent to participate in the study. Of the 339 children whose parents or guardians provided written informed consent, 88 had incomplete data (i.e., missing data for height [n = 6], body mass [n = 6], PF tests [n = 20], and/or blood tests [n = 78]). Therefore, these 88 participants were excluded from the final analysis. The study's final sample comprised 251 children aged 9–10 at baseline (125 boys and 126 girls) (Figure 1). The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical advisory committee of Tokyo Gakugei University (project identification code: 160).

Physical fitness measurements

PF was assessed by the PF test mandated by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) that is annually conducted nationwide (Ministry of Education, Culture, Sports, Science and Technology). The PF test consists of eight components and was conducted according to the test manual (Ministry of Education, Culture, Sports, Science and Technology). Detailed information on each test component is described below.

Upper body muscular strength: Upper body muscular strength was measured by the handgrip strength test using a hand-held dynamometer (Takei Scientific Instruments Co., Ltd). A previous study reported the acceptable inter-trial reliability for the hand dynamometer (Ortega et al., 2008a). The participants were asked to adjust the

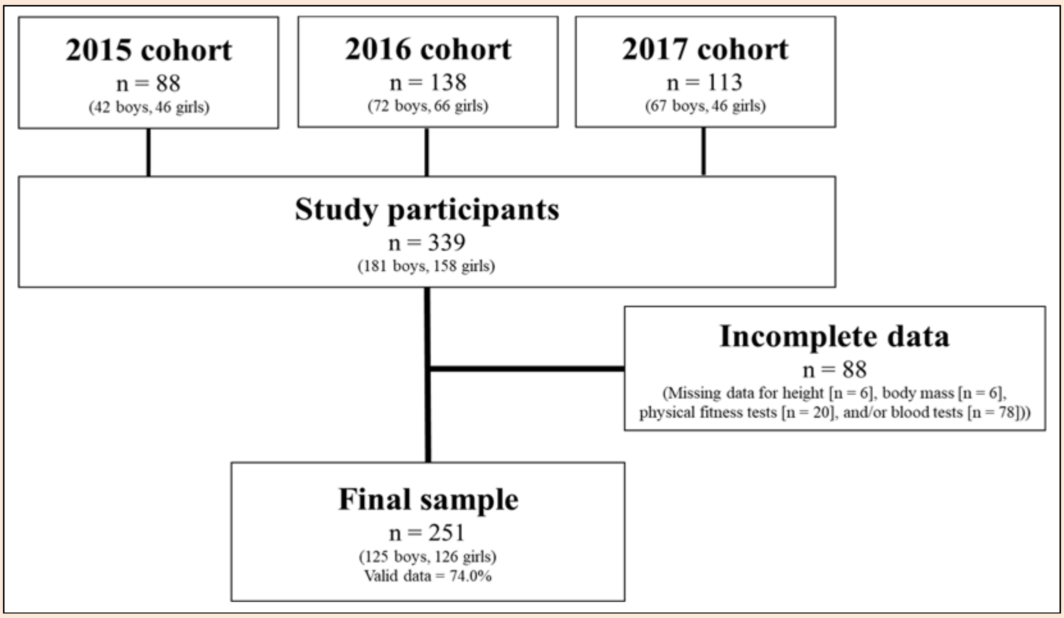

Figure 1. A flow chart for recruiting participants.

handgrip bar so that the second joint of the fingers were bent to grip the handle of the dynamometer. The participants stood upright with their arm in a vertical position and the dynamometer close to the body. They were then asked to squeeze the hand dynamometer as hard as possible. The test was completed twice each in the left and right arms, and the best recordings of both the arms were averaged.

Muscular endurance: Muscular endurance was measured using the bent-leg sit-up test. Participants were seated on a mat keeping the back straight, arms crossed in front of the chest, knees bent at a 90-degree angle with the feet flat on the mat. Participants laid backward with the back touching the mat before returning to the sitting position with elbows out in front to touch the knees. The score consisted of the number of correctly performed complete sit-ups in 30 seconds.

Flexibility: Flexibility was measured by the sit-and-reach test using a trunk flexion meter (Takei Scientific Instruments Co., Ltd). Participants sat on the floor with their legs stretched out straight in front of the body. They put both hands on the trunk flexion meter and slowly flexed forward. The test was conducted twice, and the best score was retained.

Agility: Agility was measured using the side-step test. The test was performed on a non-slip hard surface. The participants stood at a centerline, then moved 100 cm to the side (e.g., right) and touched or crossed a line with the closest foot, moved back to the center, then moved 100 cm to the other side, then back to the center. Each complete movement was recorded as 1, and the score was evaluated as the number of correctly performed complete side-steps in 20 seconds. The test was conducted twice, and the best score was retained.

Endurance performance: Endurance performance was measured using the 20-meter shuttle run test. A previous study demonstrated strong to very strong test-retest reliability and moderate to strong validity for the 20-meter shuttle run test (Tomkinson and Olds, 2008). The participants were asked to run back and forth over a 20 m distance with a progressive increase in running pace, which was controlled by pre-recorded pace-music and instructions, until fatigued (i.e., failure to keep up with the running speed twice). Several running laps for practice were provided before the test trial. The maximum number of running laps that the participants completed in the test trial were recorded as their performance on the test.

Speed: The 50-meter sprint test measured maximum running speed. Participants were instructed to run as quickly as possible between marker cones spanning over 50 meters. Only one attempt was allowed, and the time was evaluated in 0.1-second units.

Lower body muscular strength: Lower body muscular strength was measured using the standing long jump test. The test was performed on a non-slip hard surface. Participants were asked to jump the longest distance possible from a standing start swinging both arms. Distance from the take-off line to the point where the back of the heel touched the surface was measured using a measuring tape. The test was conducted twice, and the best score was retained.

Explosive arm strength and throwing ability: Explosive arm strength and throwing ability was measured by a softball throw. The participant was instructed to throw a softball with one hand (the child chooses which hand) as far as possible. Two attempts were allowed, and the best score was retained. The test item score is the distance thrown (measured in meters).

Fasting blood lipid concentrations

An overnight fasting blood venous sample was taken in the morning by doctors and nurses. Serum total cholesterol (TC) and triglyceride (TG) concentrations were measured using enzymatic methods, and high-density lipoprotein cholesterol (HDL-C) was measured using the direct measurement method with an automatic analyzer (LABOSPECT008, Hitachi High-Technologies Corporation, Japan). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula (Friedewald et al., 1972). Clustered lipid score was calculated by summing up gender- and age-specific z-scores for three lipid outcomes (TG, HDL-C, and LDL-C) as these outcomes are used as a diagnostic criterion for dyslipidemia in Japan (Teramoto et al., 2013). Z-score of HDL-C was multiplied by -1 because higher HDL-C is known to be preferable, while this is the opposite for TG and LDL-C concentrations. Additionally, we calculated non-HDL-C concentration as evidence suggests that childhood non-HDL-C concentrations are associated with cardiovascular risk factors in adults (Srinivasan et al., 2006). Non-HDL-C was calculated by subtracting the concentration of HDL-C from that of TC.

Anthropometry assessments

Body mass was measured using a digital weighing scale accurate to the nearest 0.1 kg, and height was measured using a stadiometer accurate to the nearest 0.1 cm. Body mass index (BMI) was calculated as body mass (kg) divided by height in meters squared (m^2). BMI standard deviation score (BMI-SDS) was calculated from the Japanese population-based reference values (Inokuchi et al., 2006).

Data analysis

To examine the changes in PF (i.e., baseline, 1-year follow-up, and 2-year follow-up), one-way repeated measures analysis of variance (ANOVA) were performed. When a significant difference was detected, the values were subsequently analyzed using a Bonferroni multiple comparisons test. To examine the changes in blood lipid concentrations (i.e., baseline and 2-year follow-up), paired t-tests were performed. Non-parametric tests (i.e., Wilcoxon signed-rank or Friedman's test) were used if the data were not normally distributed. For the longitudinal analysis, multilevel linear regressions were performed with a change in blood lipid concentrations over the 2-year period as the main outcome. This multilevel method enabled a two-level model to adjust for the clustered design (children within cohorts and schools) using cohorts and schools as random factors. Here, we examined whether changes in PF over the 1-year period (independent variable) was associated with the change in blood lipid concentrations over two years (dependent variable) after adjusting for baseline age, baseline BMI-SDS, changes in BMI-SDS over 1-year (i.e., ΔBMI-SDS), a baseline corresponding blood lipid concentration (e.g., when the change in non-HDL-C concentration was modeled as the dependent variable, the analysis was adjusted for the baseline non-HDL-C concentration) and the baseline corresponding PF (e.g., when the change in handgrip strength was modeled as the independent variable, the analysis was adjusted for the baseline handgrip strength). Changes in PF and blood lipid concentrations (i.e., ΔPF and Δblood lipid concentration) were determined by subtracting the values at the baseline from the values at the follow-up. The sample size was estimated based on a previous study that examined associations of cardiorespiratory fitness and muscular fitness with blood lipid concentration in Japanese children (Kidokoro and Miyashita, 2020). To detect associations between PF and blood lipid concentration with 80% power at an alpha level of 5%, we determined that a sample size of 136 participants was required.

Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 24.0. (IBM Corporation, Armonk, New York, USA), and a p-value < 0.05 denoted statistical significance.

Results

Changes in physical fitness and blood lipid concentrations over the 2-year period

All performances of physical fitness tests at the 2-year follow-up were higher than those at baseline for boys and girls. For the blood lipid concentrations, TG concentration at the 2-year follow-up was significantly higher than TG concentration at baseline for boys and girls. In contrast, HDL-C concentrations at the 2-year follow-up were significantly lower than those at the baseline for boys and girls. Additionally, LDL-C concentration at the 2-year follow-up was significantly lower than those at the baseline for girls (Table 1).

Initial 1-year changes in physical fitness and subsequent changes (over the 2-year) in blood lipid concentration

Table 2 shows the preceding changes (over 1-year) in physical fitness and the subsequent changes (over 2-year) in blood lipid concentrations. We found that preceding improvements in handgrip strength were negatively associated with subsequent changes in TG concentration for boys (β = -0.260, p = 0.030) and girls (β = -0.306, p = 0.017). For boys, preceding improvement in bent-leg sit-ups were negatively associated with clustered lipid score (β = -0.301, p = 0.038) and non-HDL-C (β = -0.310, p = 0.044). Additionally, preceding improvement in 50m sprinting were associated with subsequent change in non-HDL-C (β = 0.348, p = 0.006) and LDL-C (β = 0.408, p = 0.001). For girls, preceding improvements in standing long jump were negatively associated with non-HDL-C (β = -0.269, p = 0.021) and LDL-C (β = -0.275, p = 0.019) (Table 2, Supplement 1).

Simultaneous changes in physical fitness and blood lipid concentration over the two years

Table 3 illustrates the simultaneous changes in physical fitness and blood lipid concentration over the two years. There was no significant association between 2-year physical fitness changes and 2-year changes in blood lipid concentration for boys and girls (Table 3, Supplement 2).

Table 1. Characteristics of the participants.

	Boys (n = 125)			Girls (n = 126)		
	Baseline (4th grade)	1-year follow up (5th grade)	2-year follow up (6th grade)	Baseline (4th grade)	1-year follow up (5th grade)	2-year follow up (6th grade)
Basic characteristics						
Age (years)†	9.2 ±0.4	10.2 ±0.4**	11.2 ±0.4**	9.2 ±0.4	10.2±0.4**	11.2 ±0.4**
Height (cm)†	134.2 ±5.7	138.8 ±6.2**	145.3 ±7.5**	133.4 ±7.4	138.9±7.3**	145.5 ±7.3**
Body mass (kg)‡	31.4 ±6.4	34.1 ±7.5**	38.8 ±9.2**	30.8 ±6.2	34.7±7.5**	40.0 ±9.0**
BMI (kg/m2)‡	17.4 ±2.8	17.6 ±3.0	18.2 ±3.2**	17.2 ±2.3	17.8±2.7**	18.7 ±3.1**
BMI-SDS	0.27 ±1.22	0.06 ±1.34**	0.11 ±1.27	0.44 ±1.09	0.39±1.17	0.44 ±1.22
Physical fitness test						
Handgrip strength (kg)‡	14.6 ±3.5	17.1 ±4.4**	20.0 ±5.3**	13.9 ±3.1	16.7 ±4.1**	20.9 ±4.8**
Standing long jump (cm)†	143.4 ±20.4	149.0 ±18.8**	160.1 ±23.6**	135.0 ±17.1	145.1 ±20.5**	153.1 ±21.2**
Bent-leg sit up (times/30s)†	17.3 ±5.7	19.4 ±5.8**	19.8 ±6.1**	16.3 ±5.2	18.0 ±5.4**	18.9 ±5.3**
Ball throw (m)‡	19.3 ±7.3	22.9 ±7.3**	26.9 ±9.1**	12.6 ±4.6	16.1 ±5.7**	17.6 ±6.3**
50m sprinting (s)‡	9.8 ±1.1	9.6 ±1.3**	9.2 ±0.9**	10.2 ±0.9	9.8 ±1.0**	9.5 ±1.0**
Side-step (times/20s)†	41.0 ±6.5	41.7 ±9.4	47.1 ±7.4**	39.9 ±5.9	40.9 ±7.5**	43.2 ±8.1**
20 m shuttle run (times)†	40.8 ±21.5	47.0 ±23.8**	57.7 ±25.5**	30.6 ±18.2	38.1 ±20.7**	42.7 ±14.3**
Sit-and-reach (cm)†	31.6 ±7.4	29.6 ±7.6	32.6 ±7.0**	34.9 ±7.5	35.5 ±7.9	39.9 ±6.7**
Fasting blood lipid concentration						
Non-HDL-C (mg/dl)†	102.0 ±19.9	-	103.9 ±24.9	107.3 ±20.4	-	108.1 ±20.9
Total cholesterol (mg/dl)†	170.5 ±23.5	-	168.4 ±28.5	173.2 ±21.5	-	170.2 ±23.4
Triglyceride (mg/dl)††	69.1 ±45.7	-	79.0 ±56.3**	68.0 ±40.0	-	95.1 ±61.9**
HDL-C (mg/dl)†	67.9 ±12.7	-	64.6 ±12.9**	65.8 ±11.1	-	62.1 ±11.6**
LDL-C (mg/dl)†	88.0 ±18.6	-	88.0 ±22.4	93.4 ±17.7	-	88.9 ±20.0**

BMI: body mass index; BMI-SDS: body mass index-standard deviation score; PF: physical fitness; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. **p < 0.05 (significantly different from baseline). † One-way repeated measures analysis of variance (ANOVA) were performed. ‡ Friedman's tests were performed. †† Wilcoxon signed-rank test was performed.

Table 2. **Preceding changes (over 1-year) in physical fitness and the subsequent changes (over 2-year) in blood lipid concentrations.**

		2 year change in blood lipid concentrations									
		Clustered lipid score		NonHDL-C (mg/dl)		TG (mg/dl)		HDL-C (mg/dl)		LDL-C (mg/dl)	
		β	p	β	p	β	p	β	p	β	p
Initial 1 year change in physical fitness											
Handgrip strength (kg)	**Boys**	-0.054	0.649	0.004	0.974	-0.260	**0.030**	-0.097	0.439	0.131	0.306
	Girls	-0.214	**0.058**	-0.156	0.235	-0.306	**0.017**	0.124	0.346	0.127	0.333
Standing long jump (cm)	**Boys**	-0.125	0.358	-0.252	0.086	-0.145	0.324	-0.054	0.715	-0.142	0.332
	Girls	0.019	0.850	-0.269	0.021	0.075	0.520	-0.211	0.073	-0.275	**0.019**
Bent-leg sit up (times)	**Boys**	-0.301	**0.038**	-0.310	**0.044**	-0.151	0.331	0.089	0.564	-0.171	0.259
	Girls	0.061	0.611	-0.113	0.382	0.105	0.410	-0.152	0.242	-0.163	0.160
Ball throw (m)	**Boys**	-0.046	0.717	-0.083	0.549	-0.047	0.727	-0.049	0.719	-0.072	0.600
	Girls	-0.052	0.655	-0.138	0.296	-0.128	0.315	-0.110	0.401	-0.046	0.720
50m sprinting (s)	**Boys**	0.057	0.642	0.348	**0.006**	-0.155	0.262	0.156	0.208	0.408	0.001
	Girls	0.181	0.074	0.227	**0.053**	0.066	0.571	-0.097	0.410	0.089	0.449
Side-step (times)	**Boys**	-0.158	0.282	-0.102	0.503	-0.224	0.129	0.026	0.868	0.018	0.902
	Girls	-0.012	0.932	0.031	0.833	0.099	0.504	0.130	0.390	-0.049	0.726
20 m shuttle run (times)	**Boys**	-0.128	0.406	0.038	0.823	-0.057	0.715	0.226	0.134	-0.027	0.867
	Girls	-0.094	0.430	-0.163	0.224	0.009	0.945	0.050	0.711	-0.159	0.233
Sit-and-reach (cm)	**Boys**	-0.257	0.091	-0.125	0.435	-0.150	0.328	0.266	0.110	0.008	0.960
	Girls	-0.005	0.968	-0.003	0.985	-0.126	0.394	-0.018	0.901	0.094	0.484

HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol. Data are presented as a standardized partial regression coefficient (Β). Multilevel linear regressions were used to examine changes in physical fitness over the 1-year period (independent variable) was associated with the change in blood lipid concentrations over two years (dependent variable) after adjusting for baseline age, baseline BMI-SDS, changes in BMI-SDS over the 1-year (i.e., ΔBMI-SDS), a baseline corresponding blood lipid concentration (e.g., when the change in non-HDL-C concentration was modeled as the dependent variable, the analysis was adjusted for the baseline non-HDL-C concentration) and the baseline corresponding physical fitness (e.g., when the change in handgrip strength was modeled as the independent variable, the analysis was adjusted for the baseline handgrip strength).

Table 3. **Simultaneous changes in physical fitness and blood lipid concentration over the two years.**

		2 year change in blood lipid concentrations									
		Clustered lipid score		NonHDL-C (mg/dl)		TG (mg/dl)		HDL-C (mg/dl)		LDL-C (mg/dl)	
		β	p	β	p	β	p	β	p	β	p
2 year change in physical fitness											
Handgrip strength (kg)	**Boys**	0.170	0.334	0.224	0.263	-0.032	0.843	-0.182	0.336	0.183	0.354
	Girls	0.064	0.671	-0.111	0.516	0.155	0.355	-0.086	0.613	-0.186	0.275
Standing long jump (cm)	**Boys**	0.025	0.821	0.195	0.098	-0.103	0.377	0.066	0.557	0.138	0.346
	Girls	0.070	0.456	0.094	0.373	-0.058	0.582	-0.066	0.545	0.119	0.267
Bent-leg sit up (times)	**Boys**	-0.105	0.312	-0.042	0.718	-0.098	0.389	0.103	0.342	0.024	0.828
	Girls	0.006	0.959	-0.053	0.649	0.112	0.334	0.034	0.777	-0.125	0.257
Ball throw (m)	**Boys**	-0.040	0.869	0.158	0.557	-0.268	0.298	0.075	0.764	0.282	0.289
	Girls	0.026	0.849	0.103	0.506	-0.082	0.590	0.003	0.987	0.115	0.450
50m sprinting (s)	**Boys**	-0.096	0.336	-0.070	0.534	-0.123	0.254	0.049	0.634	0.030	0.789
	Girls	0.042	0.649	0.090	0.388	-0.011	0.917	-0.017	0.868	0.088	0.398
Side-step (times)	**Boys**	0.171	0.099	0.045	0.698	0.150	0.187	-0.160	0.136	-0.042	0.716
	Girls	-0.111	0.279	-0.127	0.244	-0.047	0.669	0.060	0.595	-0.058	0.592
20 m shuttle run (times)	**Boys**	-0.235	0.230	-0.168	0.422	-0.149	0.471	0.151	0.443	-0.105	0.629
	Girls	0.002	0.992	-0.260	0.186	0.094	0.631	-0.119	0.551	-0.272	0.172
Sit-and-reach (cm)	**Boys**	0.088	0.357	0.111	0.547	-0.121	0.245	-0.020	0.847	0.167	0.510
	Girls	0.081	0.357	0.125	0.202	-0.032	0.748	-0.040	0.698	0.116	0.241

HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol. Data are presented as a standardized partial regression coefficient (Β). Multilevel linear regressions were used to examine changes in physical fitness over the 2-year period (independent variable) was associated with the change in blood lipid concentrations over two years (dependent variable) after adjusting for baseline age, baseline BMI-SDS, changes in BMI-SDS over the 2-year (i.e., ΔBMI-SDS), a baseline corresponding blood lipid concentration (e.g., when the change in non-HDL-C concentration was modeled as the dependent variable, the analysis was adjusted for the baseline non-HDL-C concentration) and the baseline corresponding physical fitness (e.g., when the change in handgrip strength was modeled as the independent variable, the analysis was adjusted for the baseline handgrip strength).

Discussion

Main findings from the present study

The present study examined the longitudinal associations between PF components (endurance performance, muscular strength/endurance, flexibility, agility, and speed) and blood lipid concentrations in children. The study demonstrated that (1) preceding improvements in handgrip strength, bent-leg sit-ups, and 50m sprinting over the 1-year period were negatively associated with subsequent changes in blood lipid concentration over the 2-year period for boys; (2) preceding improvements in handgrip strength and standing long jumps were associated with subsequent changes in blood lipid concentration for girls; and (3) there was no significant association between 2-year changes in physical fitness and 2-year changes in blood lipid concentration for boys and girls. These results suggest that preceding changes, but not simultaneous physical fitness changes, are associated with changes in blood lipid concentration in Japanese children.

Explanation of the main findings

Previous studies examining associations of different measures of physical fitness (e.g., muscular endurance, agility, flexibility, and speed) with CVD risk markers for children demonstrated that there were significant inverse associations of standing long jump, 40-meter sprint, and flaming test performance (evaluated as balance ability) with CVD risk markers, but it was not true for other physical fitness tests (handgrip strength and sit-and-reach test) (Zaqout et al., 2016). We also found that each PF performance can be differently associated with the blood lipid concentration in Japanese children. In other words, there were significant and inverse associations of handgrip strength, bent-leg sit-ups, 50-meter sprint, and standing long jump with non-HDL-C, TG, and LDL-C concentrations. However, the other PF performances (i.e., sit-and-reach, side-step, 20-meter shuttle run, and ball throw) were not longitudinally associated with the blood lipid profile. The gender differences in LDL-C can be explained by changes in body fatness, as previously described (Dai et al, 2009); however, this was not verified in the present study as we did not measure any data regarding body fatness.

Although it is difficult to exhaustively clarify how different performance measures of PF were associated with blood lipid profiles from the present study, it is worth mentioning that all PF tests that were significantly associated with blood lipid concentrations were muscular strength-related PF tests (i.e., 50-meter sprint, standing long jump, handgrip strength, and bent-leg sit-ups). These were in line with previous studies suggesting that muscular strength (including upper and lower body muscular strength) had stronger effects on cardiometabolic risks than cardiorespiratory fitness (Artero et al., 2011; Kidokoro and Miyashita, 2020), although other studies showed the opposite results (i.e., cardiorespiratory fitness had stronger effects than muscular fitness) (Ruiz et al., 2009; Steene-Johannessen et al., 2009). Specific to the Japanese population, our previous cross-sectional study with 652 Japanese children and adolescents (aged 11.0 ± 1.5) showed that muscular strength was more closely related to non-HDL-C concentration than cardiorespiratory fitness (Kidokoro and Miyashita, 2020). The results from the present study suggest the importance of muscle-strengthening physical activities. This is in line with international physical activity guidelines that recommend muscle-strengthening activities at least three days per week (Piercy et al., 2018). Furthermore, associations between the muscular strength-related PF tests (i.e., 50-meter sprint, standing long jump, handgrip strength, and bent-leg sit-ups) and blood lipid concentrations remained significant even after adjusting BMI-SDS. This is in line with previous studies demonstrating that physical fitness was significantly associated with cardiometabolic risk independent of body fatness (Klakk et al., 2014; Schmidt et al., 2016; Shang et al., 2020).

Preceding changes in PF and subsequent changes in blood lipid concentrations

The present study is the first to demonstrate that preceding changes, but not simultaneous changes in PF, can predict subsequent changes in blood lipid concentrations in children. A plausible explanation for the findings was a time-delay in the beneficial effect of improved physical fitness on CVD markers, including blood lipid markers, which was also indicated by a previous study (Martínez-Vizcaíno et al., 2020). For example, a randomized cluster intervention study with three 60 min physical activity sessions per week over eight months for 1434 children (aged 7–14 years) from 21 schools in Spain demonstrated significant improvements in physical fitness (including cardiorespiratory fitness, muscular strength, and velocity/agility) after the intervention; however, there was no significant improvement in blood pressure (Martínez-Vizcaíno et al., 2020). It might be possible that physical fitness changes are the mediator between physical activity and CVD markers, including blood lipid profiles. Indeed, a previous study illustrated that physical fitness improvements cause a positive change in subsequent physiological and behavioral factors (Larsen et al., 2015). Our results may suggest that a physiological adaptation to growth and maturation might be a potential mechanistic explanation because we found that preceding changes, but not simultaneous changes in PF, can predict subsequent changes in blood lipid concentrations in children. The originality of the present study was to examine the associations between the preceding and simultaneous change in PF and change in blood lipid profile in children. Previous longitudinal studies mainly focused on associations between baseline PF and follow-up CVD risk markers in children (Castro-Piñero et al., 2019; Fraser et al., 2016; Grøntved et al., 2015; Savva et al., 2014; Schmidt et al., 2016; Sun et al., 2014). The findings from the present study make a significant contribution to the extant literature by providing more robust evidence on the potential cause-effect relationship between PF and blood lipid concentrations.

Strengths and limitations

The present study has several strengths that support its originality. Firstly, we used different performance measures of PF tests (endurance performance, muscular strength/endurance, flexibility, agility, and speed), which are currently used in the annual surveillance of PF in Japan that is mandated for all primary and secondary schools (Ministry of Education, Culture, Sports, Science and Technology). Therefore, our findings can help educators and practitioners to utilize the annual surveillance as an indicator of blood lipid concentrations in children without blood sampling. Secondly, the present study employed multiple fitness assessments (i.e., three time points over two years), which enabled us to understand how preceding changes in PF were associated with the subsequent changes in blood lipid concentrations. This provides more robust evidence of causality.

Despite the insights provided by our study, some limitations need to be considered. Firstly, this study is limited by the relatively short follow-up period (two years) and homogeneous sample of children from a single geographic area, which limits the generalizability of our results. However, it was worth noting that all PF tests and blood lipid concentrations in our sample were not significantly different from those of the national sample (Abe et al., 2015; Ministry of Education, Culture, Sports, Science and Technology, 2016). Secondly, we did not evaluate potential covariables that might influence our results (e.g., physical activity and socioeconomic status). Particularly, the effects of physical growth and development are known to be important covariates in pediatric research, and while we did our best to adjust this influence (including statistical adjustment for changes in BMI-SDS during the assessments), this might have distorted our results. Therefore, these variables, including maturation status, should be considered in future longitudinal studies.

Conclusion

The present study demonstrates that preceding changes, but not simultaneous changes in PF, can predict subsequent changes in blood lipid concentrations in children. The results likely reflect a physiological adaptation to growth and maturation since these associations diminished in the subsequent year.

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Key points

- Improvement in performance of handgrip strength, bent-leg sit-ups, 50-meter sprint, and standing long jump can predict subsequent changes in blood lipid concentration in Japanese children.
- Regular monitoring of PF (e.g., utilizing national fitness tests) can provide important insights for the future trajectory of blood lipid concentration in children.

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Supplement 1. Preceding changes in physical fitness and subsequent changes in clustered lipid score.

		2-year change in clustered lipid score					
		Model 1		Model 2		Model 3	
		β	p	β	p	β	p
Initial 1 year change in physical fitness							
Handgrip strength (kg)	**Boys**	0.011	0.932	0.001	0.991	-0.054	0.649
	Girls	-0.202	0.092	-0.225	0.065	-0.214	0.058
Standing long jump (cm)	**Boys**	-0.177	0.150	-0.166	0.181	-0.125	0.358
	Girls	0.069	0.571	0.076	0.536	0.019	0.850
Bent-leg sit up (times)	**Boys**	-0.268	**0.048**	-0.326	0.021	-0.301	**0.038**
	Girls	0.106	0.428	0.075	0.583	0.061	0.611
Ball throw (m)	**Boys**	-0.036	0.783	-0.069	0.608	-0.046	0.717
	Girls	0.103	0.442	0.097	0.477	-0.052	0.655
50m sprinting (s)	**Boys**	-0.009	0.940	-0.016	0.899	0.057	0.642
	Girls	0.237	**0.043**	0.256	**0.032**	0.181	0.074
Side-step (times)	**Boys**	0.057	0.684	0.056	0.688	-0.158	0.282
	Girls	-0.021	0.880	0.006	0.967	-0.012	0.932
20 m shuttle run (times)	**Boys**	0.050	0.688	0.025	0.848	-0.128	0.406
	Girls	0.010	0.935	0.013	0.915	-0.094	0.430
Sit-and-reach (cm)	**Boys**	-0.226	0.096	-0.229	0.095	-0.257	0.091
	Girls	0.040	0.767	0.052	0.706	-0.005	0.968

Data are presented as a standardized partial regression coefficient (B). Multilevel linear regressions were used to examine changes in physical fitness over the 1-year period (independent variable) was associated with the change in clustered lipid score over two years (dependent variable). Model 1: non-adjusted model; Model 2: adjusted for age and baseline BMI-SDS; Model 3: adjusted for age, baseline BMI-SDS, a baseline clustered lipid score and the baseline corresponding physical fitness (e.g., when the change in handgrip strength was modeled as the independent variable, the analysis was adjusted for the baseline handgrip strength).

Supplement 2. Simultaneous changes in physical fitness and clustered lipid score over the two years.

		2-year change in clustered lipid score					
		Model 1		Model 2		Model 3	
		β	p	β	p	β	p
2 year change in physical fitness							
Handgrip strength (kg)	**Boys**	0.053	0.568	0.089	0.348	0.170	0.334
	Girls	0.020	0.826	0.003	0.974	0.064	0.671
Standing long jump (cm)	**Boys**	0.118	0.209	0.096	0.312	0.025	0.821
	Girls	0.053	0.567	0.044	0.643	0.070	0.456
Bent-leg sit up (times)	**Boys**	-0.055	0.579	-0.050	0.617	-0.105	0.312
	Girls	0.040	0.685	0.036	0.717	0.006	0.959
Ball throw (m)	**Boys**	0.068	0.480	0.082	0.393	-0.040	0.869
	Girls	0.156	0.109	0.150	0.137	0.026	0.849
50m sprinting (s)	**Boys**	0.066	0.474	0.077	0.400	-0.096	0.336
	Girls	0.118	0.200	0.120	0.197	0.042	0.649
Side-step (times)	**Boys**	0.178	0.075	0.175	0.078	0.171	0.099
	Girls	-0.012	0.904	-0.035	0.731	-0.111	0.279
20 m shuttle run (times)	**Boys**	0.039	0.681	0.010	0.917	-0.235	0.230
	Girls	0.027	0.770	0.034	0.717	-0.047	0.789
Sit-and-reach (cm)	**Boys**	0.098	0.293	0.104	0.262	0.088	0.357
	Girls	0.029	0.755	0.029	0.756	0.081	0.357

Data are presented as a standardized partial regression coefficient (B). Multilevel linear regressions were used to examine changes in physical fitness over the 2-year period (independent variable) was associated with the change in clustered lipid score over two years (dependent variable). Model 1: non-adjusted model; Model 2: adjusted for age and baseline BMI-SDS; Model 3: adjusted for age, baseline BMI-SDS, a baseline clustered lipid score and the baseline corresponding physical fitness (e.g., when the change in handgrip strength was modeled as the independent variable, the analysis was adjusted for the baseline handgrip strength).