Combined associations of cardiorespiratory fitness and grip strength with non-high-density lipoprotein cholesterol concentrations among Japanese children and adolescents

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Abstract The present study aimed to examine the associations between cardiorespiratory fitness and muscular strength (both independently and combined) and non-high-density lipoprotein cholesterol (non-HDL-C) concentrations in 652 Japanese children and adolescents (mean $\pm$ SD, 11.0 $\pm$ 1.5 years). Fasting blood tests were conducted to measure total cholesterol, HDL-C, triglyceride, and low-density lipoprotein cholesterol concentrations. Cardiorespiratory fitness was determined using the 20 m shuttle run test (SRT), and muscular strength was obtained from grip strength (GS) tests. Relative GS, which was defined as absolute GS divided by body mass index (BMI), was computed. Cardiorespiratory fitness was inversely associated with non-HDL-C after adjusting for age, gender, and BMI ($\beta = -0.102$, $p < 0.05$). However, this association was diminished after adjusting for relative GS. In contrast, relative GS was inversely associated with non-HDL-C, and this remained significant even after adjusting for age, gender, BMI, and cardiorespiratory fitness ($\beta = -0.162$, $p < 0.05$). There was a significant interactive effect (20 m SRT*relative GS) on non-HDL-C after adjusting for age, gender, BMI ($\beta = -0.122$, $p < 0.05$). Additionally, combined analysis showed that children and adolescents with both low SRT and GS demonstrate the highest non-HDL-C concentrations compared with their counterparts, suggesting that the effects of cardiorespiratory fitness and muscular strength were additive. In conclusion, enhancing muscular fitness may be a primary target through which non-HDL-C concentrations can be regulated, particularly for children and adolescents with low cardiorespiratory fitness.

Keywords : muscular fitness, aerobic fitness, dyslipidemia, youth, health

Introduction

Cardiovascular disease (CVD) is the leading cause of global mortality¹⁾. Although the clinical manifestations of atherosclerotic disease are typically not apparent until adulthood, the process of atherosclerosis originates in childhood and progresses over the life span²⁾. Non-high-density lipoprotein cholesterol (non-HDL-C), which is calculated by subtracting the concentration of high-density lipoprotein cholesterol (HDL-C) from the total cholesterol (TC) value, is now recognized as a strong predictor of CVD³⁻⁵⁾. Its major components are low-density lipoprotein cholesterol (LDL-C) and triglyceride-rich lipoprotein cholesterol, which includes intermediate-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol. Previous studies suggest that non-HDL-C is a more accurate predictor of the development

of atherosclerotic diseases than LDL-C^{5,6)}. Additionally, non-HDL-C is easy to calculate and does not require fasting⁷⁾. For these reasons, the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases now recommend the use of non-HDL-C as the second lipid management goal^{8,9)}. Furthermore, childhood non-HDL-C concentrations are associated with adult dyslipidemia and cardiovascular risk factors^{10,11)}, suggesting that regulating non-HDL-C during childhood is important to reduce the risk of future CVD events.

Physical fitness is one of the strongest predictors of individual future CVD risk factors^{12,13)}. In particular, higher cardiorespiratory fitness during childhood has been associated with a favorable cardiovascular profile at these ages¹⁴⁻¹⁶⁾ and later in life¹⁷⁾. More recently, several lines of evidence show that higher muscular fitness in childhood is associated with a healthier cardiovascular profile in adults^{18,19)}. Importantly, some studies suggest that mus-

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cular fitness is associated with CVD risk factors independent of cardiorespiratory fitness^{20,21}). However, little is known about the combined associations between cardiorespiratory fitness and muscular fitness and CVD risk factors in children and adolescents. Additionally, it is not known how cardiorespiratory fitness and muscular fitness are associated with non-HDL-C in children and adolescents. Therefore, the purpose of the present study was to determine whether cardiorespiratory fitness and muscular fitness, independently or when combined, are associated with non-HDL-C in Japanese children and adolescents.

Materials and methods

Participants. Participants were recruited from three public primary schools and one secondary school in Saku City, Nagano, Japan. Children and adolescents in grades 4–8 (aged 9–14 years) were selected for participation. The participating classes were selected by the head and assistant head teachers at their discretion. A total of 721 cross-sectional datasets were collected in 2015 ($n = 287$) and 2016 ($n = 434$), and the data from both years were combined for analyses. Measurements were conducted from April to June in both years. Parents/guardians provided written, informed consent for their children to participate in the study. 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).

Fasting blood sample. Blood tests were conducted in May 2015 and 2016 at the participating schools. An overnight fasting blood sample was taken in the morning from the antecubital vein by doctors and nurses. Serum TC and triglyceride (TG) were measured using enzymatic methods, and HDL-C was measured using the direct measurement method with an automatic analyzer (LABOSPECT008, Hitachi High-Technologies Corporation, Japan). LDL-C was estimated using the Friedewald formula²²). Non-HDL-C was calculated by subtracting the concentration of HDL-C from TC. Because gender and age are significantly associated with serum lipid profile, gender- and age-specific z scores for each outcome were calculated based on values reported by a previous nationwide study²³). Positive z scores indicate higher values (i.e., higher risk) of each outcome compared to the national sample.

Cardiorespiratory fitness. Cardiorespiratory fitness was measured using the 20 m shuttle run test (SRT). The 20 m SRT is arguably the most popular field-based assessment and estimate of cardiorespiratory fitness in children and adolescents, and it demonstrates strong-to-very strong test–retest reliability, and moderate-to-strong validity²⁴). The participants were asked to run back and forth over a 20 m distance with progressive increases in running pace,

which was controlled by pre-recorded pace-music and instruction, until fatigue (i.e., failure to keep up with the running speed twice). Several running laps for practice were provided before the test trial. The maximal number of running laps the participants completed in the test trial was recorded as their performance in cardiorespiratory fitness. Because age and gender are significantly associated with 20 m SRT levels, age- and gender-specific z scores for the SRT were calculated based on age- and gender-matched national data²⁵). Positive z scores indicate higher values (i.e., better performance) during the SRT compared to the national sample (and vice versa).

Muscular strength. Muscular strength was measured using grip strength (GS) tests with a hand-held dynamometer (Takei Scientific Instruments Co., Ltd) according to a standardized study protocol that had been used in the national fitness survey²⁶). Acceptable inter-trial reliability was reported for the hand dynamometer²⁷). The grip bar was adjusted so that the second joints of the fingers were bent to grip the handle of the dynamometer. The participants stood upright, with the arm vertical and the dynamometer close to the body. The participants were then asked to squeeze the handgrip dynamometer as hard as possible. The test was completed twice each in the left and right arms, and the best records of the left and right arms were averaged. Because GS is highly associated with body size, calculating “relative GS” was recommended to adjust the influence of body size²⁸). Therefore, relative GS, which was defined as absolute GS divided by body mass index (BMI), was computed, as this is known to be a good indicator for health outcomes^{28,29}). Thereafter, age- and gender-specific z scores for relative GS were calculated.

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. BMI was calculated as body mass (kg) divided by height in meters squared (m^2).

Statistics. To examine the differences in anthropometry assessments, physical fitness and serum blood samples between boys and girls, independent t -tests were performed. Multiple regression models were used to examine the associations of 20 m SRT and relative GS with non-HDL-C after adjusting for age and gender (Model 1). In Model 2, multiple regression models were used to examine the associations of 20 m SRT and relative GS with non-HDL-C after adjusting for age, gender, and BMI. Thereafter, we mutually adjusted exposures (i.e., cardiorespiratory fitness and muscular fitness) for one another (i.e., when cardiorespiratory fitness was modeled as the main exposure, the analysis was adjusted for muscular fitness; and when muscular fitness was modeled as the main exposure, the analysis was adjusted for cardiorespiratory fitness, Model 3).

Next, we performed combined analyses of the associations of cardiorespiratory fitness and muscular strength with non-HDL-C. Here, participants were categorized into two groups based on mean z-scores for the 20 m SRT (i.e., HighSRT and LowSRT). In addition, according to mean z-scores for relative GS, the participants were categorized into two groups (i.e., HighGS and LowGS). Thereafter, the participants were cross-tabulated into 4 (2×2) groups to examine the combined associations of CRF and GS with non-HDL-C (i.e., “HighSRT_HighGS,” “HighSRT_LowGS,” “LowSRT_HighGS,” and “LowSRT_LowGS”). Analysis of covariance was performed to examine the differences in non-HDL-C among the groups after adjusting for age, gender, and BMI.

Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 24.0. (IBM Corporation, Armonk, New York, USA), and p -value < 0.05 denoted statistical significance. Values reported are means $\pm$ standard deviation unless otherwise stated.

Results

Final sample and basic characteristics of participants.

Of the 721 children and adolescents whose parents or guardians provided written informed consent, 69 had incomplete datasets (i.e., missing data for at least one outcome: height [$n = 12$], body mass [$n = 12$], GS [$n = 34$],

20 m SRT [$n = 45$], TC [$n = 27$], TG [$n = 30$], HDL-C [$n = 32$] and LDL-C [$n = 27$]). Therefore, those 69 participants were excluded from the final analysis. The participants were all Japanese. The final sample for the present study comprised 652 Japanese children and adolescents aged 9–14 years (327 boys and 325 girls; valid data = 90.4%).

The negative mean z-scores for both the 20 m SRT and GS indicated that the participants in the present study had slightly lower fitness levels compared to the national sample. In contrast, the negative mean z-score for non-HDL-C indicated that participants in this study had slightly healthier non-HDL-C compared to the national sample (Table 1).

Multiple regression analyses. After adjusting for age and gender, both 20 m SRT and relative GS were significantly associated with non-HDL-C. Additionally, there was a significant interactive effect (20m SRT*Relative GS) on non-HDL-C (all $p < 0.05$) (Table 2, Model 1). After additionally adjusting for BMI, there was a significant association between the 20 m SRT and non-HDL-C (Model 2, $\beta = -0.102$, $p < 0.05$). Additionally, the 20 m SRT was significantly associated with HDL-C (Model 2, $\beta = -0.136$, $p < 0.05$). However, the significant association between the 20 m SRT and non-HDL-C were diminished after additionally adjusted for relative GS (Model 3, $p >$

Table 1. Characteristics of the participants

	All ($n = 652$)		Boys ($n = 327$)		Girls ($n = 325$)		p
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	11.0	1.5	11.0	1.5	11.0	1.5	0.835
Height (cm)	145.2	11.0	145.4	11.5	144.9	10.5	0.029
Body mass (kg)	39.0	9.9	39.1	10.3	38.9	9.6	0.160
Body mass index (kg/m^2)	18.2	3.0	18.2	3.0	18.3	3.1	0.691
20 m Shuttle run (times)	53.3	26.9	60.6	29.8	45.9	21.2	< 0.001
Grip strength (kg)	20.0	7.0	20.6	7.6	19.4	6.1	< 0.001
Relative grip strength	1.10	0.34	1.14	0.38	1.06	0.28	< 0.001
Non-HDL-C (mg/dl)	103.2	22.2	100.1	21.1	106.2	23.0	0.356
Total cholesterol (mg/dl)	166.8	24.7	164.3	23.5	169.2	25.7	0.231
Triglycerides (mg/dl)	72.4	44.6	69.3	46.2	75.5	42.9	0.513
HDL-C (mg/dl)	63.5	11.6	63.9	11.9	62.4	11.0	0.199
LDL-C (mg/dl)	88.7	20.8	86.0	19.2	91.3	22.0	0.001
Z-scores							
z-20 m Shuttle run	-0.33	1.07	-0.37	1.10	-0.29	1.05	0.294
z-grip strength	-0.20	1.06	-0.27	0.99	-0.12	1.13	0.048
z-non-HDL-C	-0.14	0.89	-0.17	0.88	-0.11	0.91	0.490

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

§Relative grip strength was computed as absolute grip strength divided by body mass index

Table 2. Independent associations between the 20 m shuttle run test and relative grip strength and serum lipid profile in Japanese children and adolescents

	Non-HDL-C	TC	TG	HDL-C	LDL-C
Model 1					
20 m SRT	-0.139* (-0.235, -0.042)	-0.008 (-0.110, 0.093)	-0.182* (-0.279, -0.085)	0.238* (0.141, 0.332)	-0.059 (-0.155, 0.038)
Relative GS	-0.203* (-0.286, -0.098)	-0.124* (-0.216, -0.023)	-0.177* (-0.265, -0.073)	0.126* (0.024, 0.220)	-0.131* (-0.230, -0.030)
20 m SRT*Relative GS	-0.191* (-0.267, -0.117)	0.019 (-0.058, 0.096)	-0.149* (-0.224, -0.072)	0.157* (0.080, 0.233)	0.006 (-0.068, 0.080)
Model 2					
20 m SRT	-0.102* (-0.202, -0.001)	-0.025 (-0.122, 0.073)	-0.101 (-0.202, 0.001)	0.136* (0.037, 0.232)	-0.059 (-0.161, 0.043)
Relative GS	-0.170* (-0.260, -0.061)	-0.153* (-0.251, -0.046)	-0.087 (-0.183, 0.017)	0.007 (-0.097, 0.111)	-0.139* (-0.243, -0.031)
20 m SRT*Relative GS	-0.122* (-0.231, -0.014)	-0.064 (-0.173, 0.045)	-0.101 (-0.208, 0.006)	0.100 (-0.004, 0.208)	-0.081 (-0.191, 0.027)
Model 3					
20 m SRT	-0.048 (-0.158, 0.061)	0.037 (-0.069, 0.143)	-0.078 (-0.186, 0.029)	0.162* (0.057, 0.269)	-0.011 (-0.114, 0.094)
Relative GS	-0.162* (-0.260, -0.046)	-0.177* (-0.282, -0.061)	-0.067 (-0.171, 0.044)	-0.056 (-0.162, 0.053)	-0.142* (-0.254, -0.026)

Data are shown as standardized partial regression coefficient (95% confidence interval).

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides. Multiple regression models were performed to examine the independent associations between the 20 m shuttle run test and serum lipid profile, and between relative grip strength and serum lipid profile after adjusting for age and gender (Model 1). Model 2 was adjusted for age, gender, and body mass index. In Model 3, the exposures were mutually adjusted for one another (i.e., when cardiorespiratory fitness was modeled as the main exposure, the analysis was adjusted for muscular fitness; and when muscular fitness was modeled as the main exposure, the analysis was adjusted for cardiorespiratory fitness).

* $p < 0.05$

0.05). For relative GS, there was a significant association between relative GS and non-HDL-C after adjusting for age, gender, and BMI (Model 2, $\beta = -0.170$, $p < 0.05$). In addition, relative GS was significantly associated with TC and LDL-C (Model 2, $\beta = -0.153$ and -0.139 , both $p < 0.05$). The associations among relative GS and non-HDL-C, TC and LDL remained significant even after adjusting for the 20 m SRT (Model 3, all $p < 0.05$). There was a significant interactive effect (20 m SRT*relative GS) on non-HDL-C even after adjusting for age, gender, and BMI (Model 2, $\beta = -0.122$, $p < 0.05$).

Associations between cardiorespiratory fitness, muscular strength, and non-HDL-C. Combined analyses (20 m SRT $\times$ GS) revealed significant differences in non-

HDL-C among the four groups after adjusting for age, gender, and BMI ($F_{(3, 646)} = 3.91$, $p = 0.009$) (Fig. 1). Post-hoc tests (i.e., adjusted for multiple comparisons using the Bonferroni method) showed that the participants in the “LowSRT_LowGS” group demonstrated higher non-HDL-C concentrations than participants in the other three groups (all $p < 0.05$). There were no significant differences in non-HDL-C concentrations among the other three groups (“HighSRT_HighGS,” “HighSRT_LowGS,” “LowSRT_HighGS”) ($p > 0.05$).

Discussion

The present study demonstrated that muscular strength was significantly associated with non-HDL-C in Japa-

Fig. 1 Non-high-density lipoprotein cholesterol (non-HDL-C) concentrations among the four groups (i.e., “HighSRT_HighGS,” “HighSRT_LowGS,” “LowSRT_HighGS,” and “LowSRT_LowGS”)

Data are presented as mean $\pm$ standard error. Analysis of covariance was performed to examine the differences in non-HDL-C among the four groups after adjusting for age, gender, and BMI ($F_{(3, 646)} = 3.91$, $p = 0.009$).

* $p < 0.05$ versus “LowSRT_LowGS.”

HDL-C: high-density lipoprotein cholesterol; SRT: shuttle run test; BMI: body mass index; GS: grip strength.

nese children and adolescents. The association between relative GS and non-HDL-C remained significant even after adjusting for 20 m SRT, suggesting that muscular strength was associated with non-HDL-C independent of cardiorespiratory fitness. In contrast, although there was a significant association between cardiorespiratory fitness and non-HDL-C, the association between cardiorespiratory fitness and non-HDL-C was diminished after adjusting for relative GS. Nevertheless, there was a significant interactive effect (20 m SRT*relative GS) on non-HDL-C, suggesting that the effects of cardiorespiratory fitness and muscular strength can be additive. Additionally, our combined analysis showed that both low 20 m SRT and relative GS demonstrate the highest non-HDL-C concentrations compared to their counterparts. This suggests that higher cardiorespiratory fitness is associated with preferable non-HDL-C concentrations, regardless of relative GS. Additionally, those in LowSRT_HighGS demonstrated preferable non-HDL-C concentrations compared to those in LowSRT_LowGS. These results suggest that higher relative GS can negate the negative influences of low cardiorespiratory fitness on non-HDL-C concentrations. This finding seems to be particularly important considering that aerobic exercise can be challenging, particularly for those who have lower cardiorespiratory fitness such as overweight and obese individuals³⁰. In this respect, enhancing muscular fitness by performing resistance training may represent a more acceptable mode of exercise for those children and adolescents³⁰.

The present study shows that only muscular fitness, not cardiorespiratory fitness, was independently associated with non-HDL-C, which is contrary to previous findings³¹. For example, Steene-Johannessen et al. suggested

that both muscular fitness and cardiorespiratory fitness are independently associated with metabolic risk in children and adolescents³¹. One of the reasons for the null results for cardiorespiratory fitness in the present study may be the higher cardiorespiratory fitness in our sample. Although the cardiorespiratory fitness of our sample was equivalent to the Japanese national sample, the 20 m SRT performance of our participants was equivalent to the 90th percentile of the international norm for the 20 m SRT³². Indeed, Japanese children and adolescents appear to have one of the highest performance levels of 20 m SRT across the globe (i.e., 4th place in the world and 1st place in Asia)³³. In contrast, the GS of our sample is equivalent to counterparts from other countries³⁴. Given these results, it seems that GS is a more important determinant of non-HDL-C concentrations in Japanese children and adolescents who generally have higher cardiorespiratory fitness.

The mechanism underlying the favorable association between muscular fitness and non-HDL-C is unclear. It has been suggested that muscular fitness is significantly, but moderately associated with cardiorespiratory fitness³⁵, which we confirmed in the present study (Pearson's correlation, $r = 0.380$, $p < 0.001$). This indicates that muscular fitness may regulate non-HDL-C at least partially through a different biological pathway from cardiorespiratory fitness. Indeed, our study shows that cardiorespiratory fitness and muscular fitness are differently associated with the sub-lipid items. In other words, cardiorespiratory fitness was significantly and positively associated with HDL-C concentrations, whereas GS was significantly inversely associated with LDL-C concentrations which, in turn, resulted in lower non-HDL-C concentrations for both outcomes. It is acknowledged that

the 20 m SRT can be a good indicator of the frequency of daily aerobic activities³⁶⁾, while GS can be a good indicator of the frequency of daily muscle-strengthening activities³⁷⁾. Therefore, the differences in the associations of cardiorespiratory fitness and muscular fitness with non-HDL-C might be due to the differences in the types of exercise (i.e., aerobic or resistance exercise) the participants engaged in daily life. It has been suggested that regular aerobic exercise modestly increases HDL-C concentrations³⁸⁾. For example, a meta-analysis of 25 randomized controlled trials examining the effect of exercise training on HDL-C concentrations suggested that every 10 min prolongation of exercise per session was associated with an approximate 1.4 mg/dL (0.036 mmol/L) increase in HDL-C concentrations³⁸⁾. In contrast, it was reported that significant decreases in TC and LDL-C were found after 14 weeks of resistance training³⁹⁾. It was speculated that regular resistance training can increase fat-free mass and reduce fat mass, which are associated with decreased TC and LDL-C⁴⁰⁾. Furthermore, previous studies suggest that muscular fitness is significantly associated with insulin sensitivity⁴¹⁾, inflammatory proteins⁴²⁾ and cardiometabolic risk³¹⁾ in children and adolescents. Taken together, the present study demonstrates the importance of regular muscle strengthening activities in children and adolescents, which is consistent with international physical activity guidelines recommending muscle strengthening activities at least 3 days per week in addition to aerobic exercise in this population⁴³⁾.

The present study has a number of strengths. To the best of our knowledge, it is the first study to examine the independent and combined associations of cardiorespiratory fitness and muscular fitness with non-HDL-C among Japanese children and adolescents. Additionally, we evaluated non-HDL-C as the main outcome, which is now recognized as a strong predictor of future cardiovascular disease^{10,11)}. The present study showed that only muscular fitness, not cardiorespiratory fitness, was independently associated with non-HDL-C, suggesting that enhancing muscular fitness may be a primary target through which non-HDL-C concentrations can be regulated. GS is appealing as a simple, quick, and inexpensive non-HDL-C screening tool for children in educational settings.

Despite these strengths, there are several limitations to be considered. Our observations are limited by the cross-sectional design of the study, and as such, causality cannot be determined. Secondly, we only recruited the participants from several schools in Saku City, which limits the generalizability of our results. However, it is worthwhile to note that the participants in the present study are comparable to the national sample as there were no significant differences in 20 m SRT, grip strength, and non-HDL-C.

Conclusion

Enhancing muscular fitness may be a primary target

through which non-HDL-C concentrations can be regulated, particularly for children and adolescents with low cardiorespiratory fitness.

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Conflict of Interests

All authors declare no conflict of interests.

Author Contributions

TK and MM conceptualized the study design and protocol. TK collected and assembled the data. TK carried out the analysis and interpretation of data and drafted the manuscript. All authors have critically reviewed, revised and approved the manuscript.

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