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Relationship between Cardiorespiratory Fitness and Non-High-Density Lipoprotein Cholesterol: A Cohort Study

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Aim: Recent studies have suggested that non-high-density lipoprotein cholesterol (non-HDL-C) may be a good marker of coronary heart disease and cardiovascular disease risk. Therefore, we investigated the relationship between cardiorespiratory fitness (CRF) and non-HDL-C.

Methods: We evaluated CRF and the incidence of high level of non-HDL-C in 4,067 Japanese men without dyslipidemia. The participants were given a submaximal exercise test, a medical examination, and questionnaires on their health habits in 1986. A cycle ergometer was used to measure the CRF and maximal oxygen uptake was estimated. The incidence of a high level of non-HDL -C (≥170 mg/dL) from 1986 to 2006 was ascertained based on the fasting blood levels. A high level of non-HDL-C was found in 1,482 participants during the follow-up. Cox proportional hazard models were used to obtain the hazard ratios (HRs) and 95% confidence intervals (CIs) for the incidence of a high level of non-HDL-C.

Results: Following age adjustment, and using the lowest CRF group (quartile I) as reference, the HRs and 95% CIs for quartiles II through IV were: 1.00 (95% CI: 0.87−1.15), 0.87 (95% CI: 0.76−1.00), and 0.70 (95% CI: 0.60−0.81), respectively (P for trend <0.001). After additional adjustment for body mass index, systolic blood pressure, smoking, alcohol intake, and family history of dyslipidemia, the HRs and 95% CIs were: 1.05 (95% CI: 0.92−1.21), 0.94 (95% CI: 0.81−1.08), and 0.79 (95% CI: 0.67−0.92), respectively (P for trend=0.001).

Conclusions: These results suggest that there is an inverse relationship between CRF levels and the incidence of a high level of non-HDL-C in Japanese men.

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Key words: Exercise test, Cholesterol, Non-high-density lipoprotein cholesterol, Epidemiology, Cohort study

Introduction

Epidemiological studies show that serum lipid levels, such as total cholesterol (TC)[1)], high-density lipoprotein cholesterol (HDL-C)[2)], low-density lipoprotein cholesterol (LDL-C)[3, 4)], and non-high-density lipo-

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protein cholesterol (non-HDL-C)[2, 5], predict the risks of developing coronary heart disease (CHD) and cardiovascular disease (CVD). Non-HDL-C refers to TC minus HDL-C, and includes cholesterol of potentially atherogenic lipoprotein particles such as LDL. Furthermore, non-HDL cholesterol includes cholesterol of triglyceride-rich lipoproteins (e.g., chylomicron, very-low-density lipoprotein) and that of remnants which are known to promote the development of atherosclerosis; thus, it represents atherogenic lipoprotein profiles that cannot be measured solely as LDL-C[2]. Recent studies have suggested that the use of non-HDL-C may be superior to that of LDL-C in predicting the risk of developing CHD[2, 6, 7]. Thus, the adequate control of non-HDL-C may be critical to prevent the development of dyslipidemia, a risk factor of atherosclerotic diseases.

Many randomized controlled trials (RCTs) have examined the association between aerobic exercise (AE) and the serum lipid profile, and several review studies have performed the meta-analyses of the RCT reported in the literature. Some of the meta-analyses of studies examining TC and HDL-C in relation to AE have demonstrated that AE lowers TC and increases HDL-C[8, 9]. However, other review studies performed meta-analyses and demonstrated that AE does not have a clear influence on TC or HDL-C[10-12]. Thus, there is no consensus on how AE influences TC or HDL-C. Similarly, two studies that performed meta-analyses of RCTs examining the association between AE and non-HDL-C reported inconsistent results[13, 14]. A meta-analysis examining the relationship between the levels of non-HDL-C and walking, a moderate intensity AE, reported that AE lowers non-HDL-C levels[13]. On the other hand, another meta-analysis of RCT investigating the influence of AE and diet on non-HDL-C levels indicated that there is no significant correlation between the levels of non-HDL-C and AE alone[14]. There is also no consensus on the relationship between AE and non-HDL-C. Thus, further evidence is required to determine whether AE can prevent a high level of non-HDL-C. Therefore, we used a cohort study in Japanese men to examine the relationship between the incidence of a high level of non-HDL-C and AE, using cardiorespiratory fitness (CRF) as an objective indicator of AE[15].

Methods

Study Participants

The aim of this prospective cohort analysis[16, 17] was to assess the relationship between CRF levels and health outcomes of Japanese men who are employees of a natural gas company in the Tokyo area in Japan. This company conducts health examinations and exercise tests annually to maintain the health of employees in accordance with the Japanese Industrial Safety and Health Law and related laws.

The study cohort included 9,221 men who underwent annual health examinations and exercise tests between April 1986 and March 2006. To conduct appropriate analyses, the exclusion criteria included not having a blood test performed ($n=385$) and a diagnosis of dyslipidemia already reported at the baseline ($n=3,909$), as defined by the diagnostic criteria of the Japan Atherosclerosis Society[18, 19] (i.e., HDL-C <40 mg/dL, LDL-C ≥ 140 mg/dL, triglyceride (TG) ≥ 150 mg/dL, or non-HDL-C ≥ 170 mg/dL). Furthermore, individuals for whom CRF levels could not be accurately measured because of their inability to carry out the exercise test for at least 4 min because of abnormal findings on their electrocardiogram (ECG) or their poor physical condition were excluded ($n=12$). Women were also excluded because of their small number ($n=487$). Of the remaining 4,428 participants, another 282 with missing data on potential confounders were excluded. Finally, an individual who underwent only one blood test during the follow-up was excluded ($n=79$). The final cohort consisted of 4,067 men aged 19 to 60 years who were followed up till 2006.

The Research Ethics Committee of the National Institutes of Biomedical Innovation, Health and Nutrition approved this study (20120821-02, August 21 2012).

Baseline Examination

The height, body weight, and blood pressure at rest of all the participants were measured during health examinations conducted in 1986. A scale was used to measure the body weight with the participants in light clothing and with shoes removed. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Blood pressure at rest was measured using an automated sphygmomanometer with the participants seated in a chair. The participants aged 35 to 40 years underwent blood testing between 1986 and 1993. In 1994 and after, blood tests were conducted on the participants aged 25, 30, 35, and ≥ 40 years. All the participants were instructed to fast for 12 h prior to blood testing. The results of the blood test in the first year were used as the baseline values. Standard laboratory procedures were used to measure the levels of TC, HDL-C, TG, and glucose. Enzymatic methods were used to measure the concentration of lipids and glucose. The non-HDL-C levels were calculated by subtracting HDL-C from TC. The Friedewald equation was used to calculate the levels of LDL-C[20]. Furthermore, a self-administered questionnaire was used to collect information on potential

confounders related to CRF and non-HDL-C, including alcohol intake (non-drinker, 1−45 g/day, ≥46 g/day), smoking habit (non-smoker, 1 to 20 cigarettes/day, ≥21 cigarettes/day), and family history of dyslipidemia (yes, no). Family history of dyslipidemia was determined by a positive response at least once in the questionnaires completed during annual health examinations conducted between 1986 and 2006.

Assessment of Cardiorespiratory Fitness

A submaximal exercise test on a cycle ergometer (Monark Exercise AB, Vansbro, Sweden) was used to measure the estimated maximal oxygen uptake, an index of CRF. The exercise test was composed of a maximum of three steps, each lasting 4 min, with increased resistance in each step. The loads for the participants in the 19 to 29, 30 to 39, 40 to 49, and 50 to 60 year age groups were 98, 86, 74, and 61 W, respectively. The heart rate was measured based on the R-R interval on an ECG. The target heart rate was set as 85% of the maximum heart rate estimated according to the 220 minus age (years). The exercise test was interrupted when abnormal ECG findings occurred or when the participants indicated that they did not feel well during the test. The maximum oxygen uptake was estimated using the Åstrand-Ryhming nomogram[21] and Åstrand age-correction factors[22].

Determination of a High Level of Non-HDL-C

We used fasting blood levels to diagnose high levels of non-HDL-C based on the Japan Atherosclerosis Society published in 2012 (non-HDL-C of ≥170 mg/dL)[18, 19]. Follow-up was discontinued for participants confirmed to have started dyslipidemia treatment before the incidence of a high level of non-HDL-C in the self-administered questionnaires at health examinations between 1986 and 2006.

Statistical Analysis

The participants were categorized into quartiles by age group (≤24, 25 to 29, 30 to 34, 35 to 39, 40 to 44, 45 to 49, and ≥50 years) based on CRF, and these were then combined into four groups (Q_1, Q_2, Q_3, Q_4) to compare physical characteristics at the baseline (1986). The continuous variables are expressed as mean, standard deviation (SD) and categorical variable are expressed as percentages, respectively. We performed an analysis of variance to compare multiple group means.

Then, Cox proportional hazards model analysis was performed with the presence or absence of a high level of non-HDL-C as the dependent variable and each potential confounder as independent variables to assess the relationship between a high level of non-HDL-C and the potential confounders of BMI, systolic blood pressure, smoking habit, alcohol intake, and family history of dyslipidemia. Next, multivariable adjusted hazard ratios (HRs) adjusted for other potential confounders and 95% confidence intervals (CI) were calculated.

Further analyses were performed to examine the relationship between the level of CRF and the incidence of a high level of non-HDL-C with the presence or absence of a high level of non-HDL-C as the dependent variable and CRF categories (quartiles) as the independent variables. Then, the age-adjusted HRs and 95% CIs were calculated. The multivariable adjusted HRs and 95% CIs were calculated further adjusting for the potential confounders of BMI (continuous variable), systolic blood pressure (continuous variable), smoking habit (3 categories), alcohol intake (3 categories), and family history of dyslipidemia (yes, no) at the baseline. To assess whether the proportional hazard hypothesis was valid, a log-minus-log plot was visually checked.

Furthermore, the relationship between the level of CRF and the incidence of a high level of non-HDL-C was assessed by sensitivity analysis with a high level of non-HDL-C defined as a non-HDL-C of ≥140 mg/dL. In addition, A sensitivity analysis that excluded populations who developed a high level of non-HDL-C within 2 years of starting follow-up was performed, because it is also possible that dyslipidemia already present at the baseline affected the values of the baseline CRF. Furthermore, we divided the participants into two groups of over 40 and under 40 years, and analyzed them. Finally, the presence of a potential effect modification was verified by including potential confounders of CRF, such as age, BMI, systolic blood pressure, alcohol intake, smoking habit, and family history of dyslipidemia, into the model to verify the presence of interactions.

SPSS Statistics version 23 (IBM-SPSS, Inc., Chicago, IL, USA) was used to perform all the statistical analyses. A two-tailed probability value of <0.05 was considered statistically significant.

Results

The mean age of the participants at the time of starting the follow-up was 36.8 (SD: 9.8) years. During the follow-up period of 48,300 man-years (median, 13 years; maximum, 20 years), high levels of non-HDL-C was observed in 1,482 participants (incidence of 30.7 cases per 1,000 man-years).

Table 1 shows the physical characteristics and lifestyle habits at the baseline in the four groups by the CRF categories. A higher CRF level tended to be associated with a lower BMI, systolic blood pressure, diastolic blood pressure, smoking habit, and the baseline

Table 1. Age-adjusted baseline characteristics and lifestyle habits at the baseline

Variable	All participants	Quartiles of age-adjusted baseline cardiorespiratory fitness levels				
		Q_1 (Lowest)	Q_2	Q_3	Q_4 (Highest)	*p* value
VO_{2max}, mL/kg/min	40.7 ± 8.6	31.8 ± 4.5	37.6 ± 3.9	42.4 ± 4.4	51.1 ± 6.9	< 0.001
Number of participants	4,067	995	1,011	1,064	997	
Age, years	36.8 ± 9.8	36.8 ± 9.9	36.6 ± 9.6	37.0 ± 9.9	36.7 ± 9.8	0.831
Body mass index, kg/m^2	22.5 ± 2.4	23.3 ± 2.7	22.6 ± 2.4	22.3 ± 2.3	21.7 ± 2.0	< 0.001
Systolic blood pressure, mmHg	127.0 ± 13.0	130.7 ± 13.5	127.6 ± 12.2	126.0 ± 12.6	123.9 ± 12.9	< 0.001
Diastolic blood pressure, mmHg	72.8 ± 8.7	75.5 ± 9.0	73.2 ± 8.2	72.1 ± 8.5	70.6 ± 8.3	< 0.001
Total cholesterol, mg/dL	178.4 ± 23.6	180.3 ± 22.8	179.2 ± 22.8	178.7 ± 23.2	175.2 ± 25.2	< 0.001
High-density lipoprotein cholesterol, mg/dL	58.6 ± 12.4	57.3 ± 12.4	57.3 ± 11.7	59.1 ± 12.6	60.7 ± 12.7	< 0.001
Low-density lipoprotein cholesterol, mg/dL	102.3 ± 21.2	104.4 ± 20.7	103.9 ± 20.3	102.4 ± 21.2	98.4 ± 22.1	< 0.001
Non-high-density lipoprotein cholesterol, mg/dL	119.7 ± 23.0	123.0 ± 22.3	121.9 ± 22.2	119.6 ± 22.8	114.5 ± 23.8	< 0.001
Triglyceride, mg/dL	87.2 ± 28.7	92.9 ± 28.1	89.8 ± 29.2	85.8 ± 28.0	80.4 ± 28.0	< 0.001
Fasting blood glucose, mg/dL	93.4 ± 11.8	94.3 ± 11.8	94.0 ± 12.6	93.4 ± 11.5	92.0 ± 11.4	0.001
Smoking status, %						
None	39.3	35.8	37.2	40.8	43.5	
1-20 cigarettes /day	35.7	36.3	35.2	35.4	36.0	
≥ 21 cigarettes /day	24.9	27.9	27.6	23.8	20.5	
Alcohol intake, %						
None	29.2	29.4	29.5	28.8	29.1	
1-45 g/day	65.7	63.9	65.2	67.0	66.6	
≥ 46 g/day	5.1	6.6	5.3	4.2	4.3	
Family history of dyslipidemia, %	2.9	2.9	3.2	2.3	3.2	

Data are the means ± SD or percentages.
VO_{2max}, maximal oxygen uptake

lipid levels. However, there were no clear differences in the alcohol intake or family history of dyslipidemia among the CRF categories.

Table 2 shows the adjusted multivariable HRs adjusted for incidence of a high level of non-HDL-C and 95% CIs assessed by potential confounders. There was a positive dose-response relationship between the incidence of a high level of non-HDL-C and BMI and a negative dose-response relationship with the amount of alcohol consumed. The participants with a family history of dyslipidemia had higher HRs for the incidence of a high level of non-HDL-C. In contrast, there was no clear relationship between the incidence of a high level of non-HDL-C and age, systolic blood pressure, or smoking habit.

Table 3 shows the multivariable adjusted HRs and 95% CIs of the incidence of high levels of non-HDL-C by CRF levels categorized by quartile. There was a significant negative dose-response relationship between the adjusted multivariable HRs of Q_2, Q_3, and Q_4 CRF based on Q_1. We further adjusted for HDL-C and TG because the baseline lipid levels were different between the CRF categories.

Thus, we adjusted HDL-C and TG. As we expected, further adjustment for HDL-C and TG at the baseline examination substantially attenuated the HRs. The multivariable hazard ratio was Q_1 = 1.00, Q_2 = 1.06 (95% IC, 0.92-1.22), Q_3 = 1.01 (95% IC, 0.99-1.14), Q_4 = 0.86 (95% IC, 0.73-1.00), (*p* for trend = 0.040). HDL-C and TG are likely to be intermediate factors for CRF; thus, this result may not indicate the effect of CRF accurately. Furthermore, the optimal management goal of non-HDL-C has been discussed[23-25]; the same results as those in the main analysis were obtained by sensitivity analysis that examined the relationship between CRF levels and the incidence of a high level of non-HDL-C defined as a non-HDL-C level of ≥ 140 mg/dL, instead of ≥ 170 mg/dL ($p < 0.001$) (**Supplementary Table 1**). The same results as those in the main analysis were obtained by sensitivity analysis that excluded the participants with a high level of non-HDL-C within 2 years of the baseline ($p = 0.014$) (**Supplementary Table 2**). In addition, for the analysis in which the age of the participants was divided into two groups, the same results as the main analysis were attained (**Supplementary Table 3**). Finally, no significant interactions were observed between the CRF levels and age, BMI, systolic blood pressure, alcohol intake, smok-

Table 2. Multivariable adjusted hazard ratios for incidence of a high level of non-HDL-C by potential risk factors

Potential risk factors	Participants	Number of cases	Incidence rate §	Hazard ratio (95% CI)	*p* value
Age (single year)	4.067	1.482	30.7	1.01 (1.00−1.01)	0.015
Body mass index					
1st tertile (Low)	1.362	412	23.4	1.00 (Reference)	−
2nd tertile (Middle)	1.357	498	30.4	1.27 (1.11−1.45)	<0.001
3rd tertile (High)	1.348	572	39.9	1.58 (1.38−1.80)	<0.001
Sytolic blood pressure					
<140 mmHg	3.474	1.264	29.9	1.00 (Reference)	−
≥140 mmHg	593	218	35.9	1.06 (0.92−1.23)	0.425
Smoking status					
None	1.600	592	32.0	1.00 (Reference)	−
1-20 cigarettes/day	1.453	506	28.7	0.96 (0.85−1.09)	0.524
≥21 cigarettes/day	1.014	384	31.6	1.02 (0.90−1.16)	0.754
Alcohol intake					
None	1.187	440	30.7	1.00 (Reference)	−
1-45 g/day	2.672	971	30.8	0.95 (0.85−1.07)	0.424
≥46 g/day	208	71	28.8	0.84 (0.65−1.08)	0.173
Family history of dyslipidemia					
No	3.949	1.412	30.1	1.00 (Reference)	−
Yes	118	70	53.3	1.85 (1.45−2.35)	<0.001

CI, confidence interval
Adjusted for cardiorespiratory fitness level and all items in the table.
§Incidence rate is presented in cases per 1,000 man-years of observation.

ing habit, or family history of dyslipidemia (all $p \geq 0.10$).

Discussion

In the present study, we examined the association between CRF, an indicator of AE[15], and the incidence of a high level of non-HDL-C in a cohort of Japanese men who were followed for a period of 13 years (median). Our results demonstrated that Japanese men with a high CRF level at the baseline had a lower incidence rate of a high level of non-HDL-C. This suggests that habitual AE may reduce the risk of having a high level of non-HDL-C.

To our knowledge, no study has investigated the association between CRF and the incidence of a high level of non-HDL-C. However, many RCTs have investigated the effect of AE on the serum lipid profile, and these studies have been examined collectively in meta-analyses. Kelly *et al.*[13] reviewed 22 RCTs that examined the influence of walking, a moderate intensity AE, on the levels of TC and HDL-C, and demonstrated that AE reduces non-HDL-C levels by 4%. This study suggests that moderate intensity AE may prevent a high level of non-HDL-C. An RCT by O'Donovan *et al.*[26] investigated the effect of moderate and high-intensity AE on the levels of non-HDL-C in sedentary men, and demonstrated a significant dose-response relationship between the intensity of exercise and levels of non-HDL-C reduction. In agreement with this study, we demonstrated a significant inverse dose-response relationship between CRF and the incidence of a high level of non-HDL-C. Collectively, these results suggest that moderate intensity AE such as walking may prevent a high level of non-HDL-C, and that AE at a higher intensity may be more effective for its prevention. However, another study by Kelly *et al.*[14], involving a meta-analysis of 6 RCTs that examined the association between the levels of non-HDL-C and either AE, diet, or their combination, demonstrated that AE alone does not reduce the levels of non-HDL-C. The result may have been influenced by the fact that the study participants in the "AE alone" group for each RCT included those with lower non-HDL-C levels at the baseline compared with those in the "diet alone" and "diet + AE" groups. Thus, further studies are warranted to determine whether AE can prevent a high level of non-HDL-C.

A few plausible mechanisms may explain the results of the present study. First, physical activity activates AMP-activated protein kinase in the skeletal muscle and promotes the expression of lipoprotein lipase (LPL), an enzyme degrading TG into free fatty acids and glycerol by increasing PPARγ1 levels[27]. Accordingly, the increase in LPL activity following exercise increases the

Table 3. Multivariable adjusted hazard ratios for incidence of a high level of non-HDL-C according to cardiorespiratory fitness levels

	Quartiles of age-adjusted baseline cardiorespiratory fitness levels				*p* value for trend
	Q_1 (Lowest)	Q_2	Q_3	Q_4 (Highest)	
Number of participants	995	1.011	1.064	997	
Number of cases	390	404	384	304	
Incidence rate [§]	34.5	34.5	30.2	24.2	
Model 1 hazard ratio (95% CI)	1.00 (reference)	1.00 (0.87−1.15)	0.87 (0.76−1.00)	0.70 (0.60−0.81)	<0.001
Model 2 hazard ratio (95% CI)	1.00 (reference)	1.01 (0.88−1.16)	0.89 (0.77−1.03)	0.72 (0.62−0.84)	<0.001
Model 3 hazard ratio (95% CI)	1.00 (reference)	1.05 (0.92−1.21)	0.94 (0.81−1.08)	0.79 (0.67−0.92)	0.001

CI, confidence interval
Model 1: adjusted for age.
Model 2: Model 1 plus systolic blood pressure, smoking status, alcohol intake, and family history of dyslipidemia.
Model 3: Model 2 plus body mass index.
[§]Incidence rate is presented in cases per 1,000 man-years of observation.

rate of TG clearance, resulting in decreased TG levels[28]. The activation of LPL as, described above, causes TG-rich lipoprotein levels to decrease since LPL hydrolyzes TG in TG-rich lipoprotein particles[29]. Second, the increase in glucose transporter 4 associated with physical activity may reinforce insulin sensitivity[30]. Increased insulin sensitivity leads to decreased hepatic very-low-density lipoprotein (VLDL) and Apo B secretion, as well as decreased free fatty acids in the liver[28]. In addition, increased insulin sensitivity suppresses the exchange reaction between TG and cholesterol ester in HDL particles by cholesteryl ester transfer protein and TG-rich lipoprotein particles (VLDL particles, LDL particles). Therefore, VLDL, LDL, and small dense LDL decrease[28]. Furthermore, exercise reduces the expression of lipoprotein convertase subtilisin/kexin type 9, resulting in increased clearance of LDL-C and thus decreased LDL-C[31]. Third, increased insulin sensitivity elevates Lecithin cholesterol acyltransferase activity, suppresses cholesteryl ester transfer protein activity, and reduces hepatic triglyceride lipase activity, resulting in increased HDL_2 and decreased HDL_3[28]. Additionally, LPL activation leads to increased transport of lipids and lipoproteins into peripheral circulation and the hepatic tissues, resulting in increased HDL-C levels. As a result of these mechanisms, high non-HDL-C values may have occurred less frequently in the high CRF group in this study compared to in the group exhibiting low CRF.

To our knowledge, this is the first study describing the association between non-HDL-C levels and CRF, an objective indicator of AE and an important measure of cardiorespiratory capacity of an individual at a given degree of fitness and oxygen availability[32]. We focused on non-HDL-C as the primary outcome; since it is not influenced by the level of triglycerides, it is considered a predictive indicator of CHD[2]. Therefore, our study provides novel evidence to highlight the importance of evaluating CRF to reduce the risks of developing various diseases related to non-HDL-C, including dyslipidemia. While there have been RCTs examining the influence of AE on TC, HDL-C, and non-HDL-C levels, RCTs have been criticized for their low external validity in spite of the high internal validity. The present study is observational in nature and enrolled a large number of participants; thus, while the study design is less rigorous than an RCT is, the results may be more generalizable.

However, the present study has some limitations. First, the CRF in this study is an indirect measurement of maximum oxygen uptake, and may be less accurate than a direct measurement of maximum oxygen uptake. However, the method used in the present study, which relies on the estimation of the maximum oxygen uptake, has been validated as being highly correlated with the direct measurement of CRF[33, 34]. Second, we only measured CRF once at the baseline. However, the change in CRF during the follow-up period tends to introduce regression dilution bias; thus, the results of the present study may in fact be an underestimation, which does not reduce the internal validity. Third, we did not have information on diet, which influences lipid levels; therefore, residual confounding by diet may be present. Fourth, the lack of data on insulin resistance and glycemic markers may have biased the results through the potential effects on non-HDL-C. Furthermore, the participants were only men, limiting the generalizability of this study but not its validity. Last, the study participants were restricted to those who resided in the Tokyo area and who were working at a particular company. Therefore, they may not be representative of Japanese men. In spite of this limitation, the participants had various roles within the company, suggesting that they could represent Japanese men to a certain extent. However, further studies are needed to validate the results

of this study in non-Asian participants with a high proportion of obesity and who have different lifestyles to the Japanese.

Conclusions

In the present study, we demonstrated that Japanese men with high levels of CRF are less likely to have a high level of non-HDL-C. Our findings indicate that habitual AE may prevent a high level of non-HDL-C, a risk factor for CHD.

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Conflicts of Interests

The authors declare that they have no competing interests.

References

1) Okamura T, Tanaka H, Miyamatsu N, Hayakawa T, Kadowaki T, Kita Y, Nakamura Y, Okayama A, Ueshima H, NIPPON DATA80 Research Group: The relationship between serum total cholesterol and all-cause or cause-specific mortality in a 17.3-year study of a Japanese cohort. Atherosclerosis, 2007; 190: 216-223
2) Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, Bush TL: Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. Arch Intern Med, 2001; 161: 1413-1419
3) Imano H, Noda H, Kitamura A, Sato S, Kiyama M, Sankai T, Ohira T, Nakamura M, Yamagishi K, Ikeda A, Shimamoto T, Iso H: Low-density lipoprotein cholesterol and risk of coronary heart disease among Japanese men and women: the Circulatory Risk in Communities Study (CIRCS). Prev Med, 2011; 52: 381-386
4) Farrell SW, Finley CE, Grundy SM: Cardiorespiratory fitness, LDL cholesterol, and CHD mortality in men. Med Sci Sports Exerc, 2012; 44: 2132-2137
5) Kitamura A, Noda H, Nakamura M, Kiyama M, Okada T, Imano H, Ohira T, Sato S, Yamagishi K, Iso H: Association between non-high-density lipoprotein cholesterol levels and the incidence of coronary heart disease among Japanese: the Circulatory Risk in Communities Study (CIRCS). J Atheroscler Thromb, 2011; 18: 454-463
6) Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J: Major lipids, apolipoproteins, and risk of vascular disease. JAMA, 2009; 302: 1993-2000
7) Moriyama K, Takahashi E. Non-HDL Cholesterol is a More Superior Predictor of Small-Dense LDL Cholesterol than LDL Cholesterol in Japanese Subjects with TG Levels <400 mg/dL. J Atheroscler Thromb. 2016; 23: 1126-1137
8) Kelley GA, Kelley KS: Aerobic exercise and lipids and lipoproteins in men: a meta-analysis of randomized controlled trials. J Mens Health Gend, 2006; 3: 61-70
9) Kodama S, Tanaka S, Saito K, Shu M, Sone Y, Onitake F, Suzuki E, Shimano H, Yamamoto S, Kondo K, Ohashi Y, Yamada N, Sone H: Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: a meta-analysis. Arch Intern Med, 2007; 167: 999-1008
10) Kelley GA, Kelley KS, Tran ZV. Walking, lipids, and lipoproteins: a meta-analysis of randomized controlled trials. Prev Med, 2004; 38: 651-661
11) Kelley GA, Kelley KS, Vu Tran Z: Aerobic exercise, lipids and lipoproteins in overweight and obese adults: a meta-analysis of randomized controlled trials. Int J Obes (Lond), 2005; 29: 881-893
12) Kelley GA, Kelley KS, Roberts S, Haskell W: Comparison of aerobic exercise, diet or both on lipids and lipoproteins in adults: a meta-analysis of randomized controlled trials. Clin Nutr, 2012; 31: 156-167
13) Kelley GA, Kelley KS, Tran ZV: Walking and Non-HDL-C in adults: a meta-analysis of randomized controlled trials. Prev Cardiol, 2005; 8: 102-107
14) Kelley GA, Kelley KS: Effects of Diet, Aerobic Exercise, or Both on Non-HDL-C in Adults: A Meta-Analysis of Randomized Controlled Trials. Cholesterol, 2012; 2012: 840935
15) Ross R, Blair SN, Arena R, Church TS, Després JP, Franklin BA, Haskell WL, Kaminsky LA, Levine BD, Lavie CJ, Myers J, Niebauer J, Sallis R, Sawada SS, Sui X, Wisløff U, American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health, Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular and Stroke Nursing, Council on Functional Genomics and Translational Biology, Stroke Council: Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. Circulation, 2016; 134: e653-e699
16) Sawada SS, Muto T, Tanaka H, Lee IM, Paffenbarger RS Jr, Shindo M, Blair SN: Cardiorespiratory fitness and cancer mortality in Japanese men: a prospective study. Med Sci Sports Exerc, 2003; 35: 1546-1550
17) Sawada SS, Lee IM, Muto T, Matuszaki K, Blair SN: Cardiorespiratory fitness and the incidence of type 2 diabetes: prospective study of Japanese men. Diabetes care, 2003; 26: 2918-2922
18) Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi

S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, Yokote K, Japan Atherosclerosis Society (JAS): Comprehensive risk management for the prevention of cardiovascular disease: executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan -- 2012. J Atheroscler Thromb, 2013; 20: 603-615
19) Japan Atherosclerosis Society (JAS): Comprehensive risk management for the prevention of cardiovascular disease: executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan -- 2017. (Japanese)
20) Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 1972; 18: 499-502
21) Åstrand PO, Ryhming I: A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. J Appl Physiol, 1954; 7: 218-221
22) Åstrand I: Aerobic work capacity in men and women with special reference to age. Acta Physiol Scand Suppl, 1960; 49: 1-92
23) Tada H, Kobayashi J. Optimal Management Target for Non-High-Density Lipoprotein Cholesterol. J Atheroscler Thromb, 2016; 23: 399-401
24) Kuwabara K, Harada S, Sugiyama D, Kurihara A, Kubota Y, Higashiyama A, Hirata T, Nishida Y, Kawasaki M, Takebayashi T, Okamura T. Relationship between Non-High-Density Lipoprotein Cholesterol and Low-Density Lipoprotein Cholesterol in the General Population. J Atheroscler Thromb. 2016; 23: 477-490
25) Saiki Y, Otsuka T, Kato K, Kawada T. A Proposal for the Optimal Management Target for Serum Non-High-Density Lipoprotein Cholesterol Level in Low-Risk Japanese Workers. J Atheroscler Thromb. 2016; 23: 422-430
26) O'Donovan G, Owen A, Bird SR, Kearney EM, Nevill AM, Jones DW, Woolf-May K: Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost. J Appl Physiol (1985), 2005; 98: 1619-1625
27) Sasaki T, Nakata R, Inoue H, Shimizu M, Inoue J, Sato R: Role of AMPK and PPARgamma1 in exercise-induced lipoprotein lipase in skeletal muscle. Am J Physiol Endocrinol Metab, 2014; 306: E1085-1092
28) Després JP, Lamarche B: Low-intensity endurance exercise training, plasma lipoproteins and the risk of coronary heart disease. J Intern Med, 1994; 236: 7-22
29) Saraswathi V, Hasty AH: The role of lipolysis in mediating the proinflammatory effects of very low density lipoproteins in mouse peritoneal macrophages. J Lipid Res, 2006; 47: 1406-1415
30) Ivy JL, Zderic TW, Fogt DL: Prevention and treatment of non-insulin-dependent diabetes mellitus. Exerc Sport Sci Rev, 1999; 27: 1-35
31) Arsenault BJ, Pelletier-Beaumont E, Alméras N, Tremblay A, Poirier P, Bergeron J, Després JP: PCSK9 levels in abdominally obese men: association with cardiometabolic risk profile and effects of a one-year lifestyle modification program. Atherosclerosis, 2014; 236: 321-326
32) Levine BD: V̇O2max: what do we know, and what do we still need to know?. J Physiol, 2008; 586: 25-34
33) Cink RE, Thomas TR: Validity of the Åstrand-Ryhming nomogram for predicting maximal oxygen intake. Br J Sports Med, 1981; 15: 182-185
34) Teräslinna P, Ismail AH, MacLeod DF: Nomogram by Åstrand and Ryhming as a predictor of maximum oxygen intake. J Appl Physiol, 1966; 21: 513-515

Supplementary Table 1. Multivariable adjusted hazard ratios for incidence of a high level of non-HDL-C (≥140 mg/dL) according to cardiorespiratory fitness levels

	Quartiles of age-adjusted baseline cardiorespiratory fitness levels				*p* value for trend
	Q_1 (Lowest)	Q_2	Q_3	Q_4 (Highest)	
Number of participants	738	770	844	834	
Number of cases	530	556	560	501	
Incidence rate[§]	90.4	87.7	77.2	64.0	
Model 1 hazard ratio (95% CI)	1.00 (reference)	0.96 (0.85−1.08)	0.85 (0.76−0.96)	0.71 (0.63−0.80)	<0.001
Model 2 hazard ratio (95% CI)	1.00 (reference)	0.96 (0.85−1.09)	0.86 (0.76−0.97)	0.71 (0.63−0.81)	<0.001
Model 3 hazard ratio (95% CI)	1.00 (reference)	1.00 (0.88−1.12)	0.89 (0.79−1.01)	0.76 (0.67−0.86)	<0.001

CI, confidence interval
Model 1: adjusted for age.
Model 2: Model 1 plus systolic blood pressure, smoking status, alcohol intake, and family history of dyslipidemia.
Model 3: Model 2 plus body mass index.
[§]Incidence rate is presented in cases per 1,000 man-years of observation.

Supplementary Table 2. Multivariable adjusted hazard ratios for incidence of a high level of non-HDL-C according to cardiorespiratory fitness levels excluding those who had a high level of non-HDL-C within 2 years after the start of follow up

	Quartiles of age-adjusted baseline cardiorespiratory fitness levels				*p* value for trend
	Q_1 (Lowest)	Q_2	Q_3	Q_4 (Highest)	
Number of participants	936	960	1,017	975	
Number of cases	331	353	337	282	
Incidence rate[§]	29.5	30.3	26.6	22.5	
Model 1 hazard ratio (95% CI)	1.00 (reference)	1.03 (0.89−1.19)	0.90 (0.77−1.04)	0.76 (0.65−0.89)	<0.001
Model 2 hazard ratio (95% CI)	1.00 (reference)	1.04 (0.89−1.20)	0.91 (0.78−1.06)	0.77 (0.66−0.91)	<0.001
Model 3 hazard ratio (95% CI)	1.00 (reference)	1.08 (0.93−1.26)	0.96 (0.82−1.12)	0.84 (0.71−0.99)	0.014

CI, confidence interval
Model 1: adjusted for age.
Model 2: Model 1 plus systolic blood pressure, smoking status, alcohol intake, and family history of dyslipidemia.
Model 3: Model 2 plus body mass index.
[§]Incidence rate is presented in cases per 1,000 man-years of observation.

Supplementary Table 3. Multivariable adjusted hazard ratios for incidence of a high level of non-HDL-C according to cardiorespiratory fitness levels excluding those who had a high level of non-HDL-C (≥40, <40 age at baseline)

	Quartiles of age-adjusted baseline cardiorespiratory fitness levels				*p* value for trend
	Q_1 (Lowest)	Q_2	Q_3	Q_4 (Highest)	
≥40 age at baseline					
Number of participants	365	349	383	367	
Number of cases	130	111	125	99	
Incidence rate[§]	39.8	33.8	36.6	26.7	
Hazard ratio (95% CI)	1.00 (reference)	0.85 (0.66−1.10)	0.94 (0.74−1.21)	0.73 (0.56−0.95)	0.053
<40 age at baseline					
Number of participants	630	662	681	630	
Number of cases	260	293	259	205	
Incidence rate[§]	32.4	34.8	27.8	23.2	
Hazard ratio (95% CI)	1.00 (reference)	1.17 (0.99−1.38)	0.94 (0.79−1.13)	0.83 (0.68−1.00)	0.011

CI, confidence interval
Adjusted for age, systolic blood pressure, smoking status, alcohol intake, and family history of dyslipidemia, and body mass index.
[§]Incidence rate is presented in cases per 1,000 man-years of observation.