Comparison of Effects of Daily Physical Activity, Obesity, and Alcohol Drinking and Cigarette Smoking Habits on Serum Levels of High Density Lipoprotein Cholesterol and Apolipoprotein A-1 among Schoolchildren and Their Parents

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Quantitative and qualitative difference of the effects of life style factors ranging within the usual daily life on high density lipoprotein (HDL) were estimated in the 5th grade schoolchildren (n=155) and their parents by a cross sectional study. Strength of daily physical activity was measured directly and drinking and smoking habits, inquired by a questionnaire. By multiple linear regression analysis in the fathers, change in HDL cholesterol (HDL-C) estimated by increasing the level of strength of physical activity, body mass index, daily amount of alcohol and cigarettes from the mean of bottom tertile to that of top one was 0.08 [95% confidence interval: -0.03 to 0.19], -0.20 [-0.30 to -0.09], 0.31 [0.19 to 0.42], -0.16 [-0.27 to -0.05] mmol/liter, respectively, and that of apolipoprotein A-1 (Apo A-1), 1.1 [-2.2 to 4.4], -4.4 [-7.8 to -1.1], 9.0 [5.6 to 12.4], -2.4 [-5.8 to -0.9] μmol/liter, respectively. The ratio of HDL-C to Apo A-1 increased by high activity, weight loss, alcohol, and abstaining from smoking. Although change in HDL-C and Apo A-1 due to alcohol was greatest, estimated HDL-C change accompanying a unit of estimated concentration change of Apo A-1 was smallest. These results suggested variability of HDL change in terms of its subfractional constitution, and changes induced by alcohol seemed less desirable than that induced by the other factors. Regression was borderline or insignificant in the children and mothers, who did not smoke or drink.

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MATERIALS AND METHODS

Materials

Materials were all the 155 fifth-grade schoolchildren in the years of 1988 and 1989 and their parents living in a small agricultural village in Niigata prefecture. They were determined for their serum concentrations of HDL-C and Apo A-1, energy expenditure by daily physical activity, body size, and alcohol drinking and cigarette smoking habits. The numbers of the fathers and mothers from whom complete data set was obtained were 124 and 145, respectively. Fourteen mothers who had either or both of drinking habits were eliminated from the analysis because of their small number.

Data Acquisition and Chemical Analysis

Body mass index (BMI), (body weight (kg)/square of body length (m) determined at blood sampling), was used as an index for obesity. Blood sample of the schoolchildren was taken before noon and that of the parents, taken in the morning after overnight fasting in September of each year. HDL-C was separated from chylomicrons, low density lipoprotein cholesterol and very low density lipoprotein cholesterol by phosphotungstate magnesium method. Serum concentrations of total cholesterol and HDL-C were determined enzymatically with an autoanalyzer VP Super System (Abbott Laboratories, Abbott Park, Illinois), and the remaining sera were stored at −80°C to be determined for their concentration of Apo A-I with an autoanalyzer Cobas Mira (F. Hoffman-La Roche & Co, Basel, Switzerland) by an immunoturbidimetry method six months later. The molecular weight of Apo A-I to convert to SI unit was 28,171 based on the report by Brewer et al 24).

Since the chemical determination was done in separate years, its reliability was checked by pooled sera prepared at the Department of Laboratory Medicine of Niigata University. The means and standard deviations of HDL-C of the pooled sera determined in the respective survey years were 0.61 ± 0.01 mmol/liter (n = 5) and 0.65 ± 0.06 mmol/liter (n = 7), and those of Apo A-1 were 47.5 ± 2.9 μmol/liter (n = 15) and 47.4 ± 1.6 μmol/liter (n = 12). Coefficient of variation throughout the two years was 9.0 percent for HDL-C and 5.0 percent for Apo A-1.

The energy expenditure by daily physical activity was determined two consecutive days excluding weekend and holidays during early November and early December of the same year for every subject by Calorie Counter (Suzuken Co. Ltd., Nagoya, Japan), a commercially available portable device. The device sensed acceleration of body movements to convert it to the value of energy in kcal by an empirical equation (Energy = K • Body weight, where K is a preset constant with ten grades experimentally determined by the manufacturer to reflect acceleration) for every time interval of three seconds. The energy thus obtained was summed up for the time interval during which the subject carried the device and displayed on a window by digital numbers. The ability of this device to determine energy has been tested good by treadmill exercise test 25).

Every subject filled out the form which asked the number of cigarettes do you smoke a day on the average? The choices and scores of the answer were: none: 0, ten or less: 5, 11 to 20: 15, 21 to 40: 30, and 41 or more: 50.

To check consistency of Sake drinking with general drinking habit, the subjects were examined for the kind and amount of alcoholic beverages they took during two consecutive days in October by dietitians. The total energy measured for two days was averaged and divided by body weight, and this value was used in this study so that mean strength rather than total amount of physical activity could be related to changes of HDL constituents.

Drinking and smoking habits were inquired by a questionnaire having categorical answers and allocated scores as follows. “How much do you drink Sake (a traditional Japanese alcoholic beverage brewed from rice) a day on the average?” The choices and scores of the answer were: none: 0, less than one unit: 0.5, one to less than two units: 1.5, two to less than three units: 2.5, and three or more units: 3.5, where one unit, a traditional measure of fluid volume equal to 180 ml, of Sake contained about 22 g of ethanol. “How many numbers of cigarette do you smoke a day on the average?” The choices and scores of the answer were: none: 0, ten or less: 5, 11 to 20: 15, 21 to 40: 30, and 41 or more: 50.

To check consistency of Sake drinking with general drinking habit, the subjects were examined for the kind and amount of alcoholic beverages they took during two consecutive days in October by dietitians. The correlation coefficient between the scores of categorical answers and the amount of ethanol taken from any kind of alcoholic beverages during the survey was 0.57 (p < 0.001) in 116 fathers out of 124.

STATISTICAL ANALYSIS

To estimate the effect of each of alcohol drinking, cigarette smoking, physical activity and BMI on HDL-C and Apo A-1 by adjusting confounding between these factors, first, multiple regression equation was obtained by using serum levels of HDL-C or Apo A-1 as the dependent variable and scores of categorical answers or measured values of the four life style factors as the explanatory variable. In the children and mothers, who had no smoking or drinking habits, only strength of physical activity and BMI were used as the explanatory variable.
explanatory variable. Age of the subjects was not included as an explanatory variable since most parents were within the short age-range between late thirties and early forties.

Then, to make the effects due to the above four factors which have different measurement units comparable under the same condition, change of HDL-C and Apo A-1 to be produced when the factor under consideration was changed from the mean of the bottom tertile subgroup to that of the top tertile one was obtained as the difference of the expected values between the respective tertile subgroups which were calculated through the multiple regression equation determined above. In this calculation, for the values of factors which were left out of consideration were entered the respective means of whole fathers, mothers or children.

**RESULTS**

The means and standard deviations of the variables of the subject groups are shown in Table 1. Strength of physical activity was highest in the children and lowest in the mothers. BMI was highest in the fathers and lowest in the children. HDL-C was highest in the children and lowest in the fathers. Although Apo A-1 was highest in the fathers and lowest in the children, difference of Apo A-1 levels between the subject groups was smaller than that of HDL-C.

Correlation coefficient (R) of HDL-C and Apo A-1 in 93 triplets within the whole subjects is shown in Table 2. Correlation coefficient of HDL-C was significant between mothers and children (R = 0.41, p < 0.01) but was insignificant both between fathers and children and between the parents. Apo A-1 took a positive, borderline significant correlation between children and either of the fathers and mothers (R = 0.19, p < 0.10) but did not show a significant correlation between the parents.

When the multiple linear regression analysis was applied, significant relationship of the life style factors with HDL-C (coefficient of determination (R²) = 0.29, p < 0.01) and with Apo A-1 (R² = 0.23, p < 0.01) was obtained for the fathers, and borderline relationship with HDL-C (R² = 0.03, p < 0.1) and with Apo A-1 (R² = 0.04, p < 0.1) was obtained for the children as shown in Table 3. However, there was no significant or even borderline relationship for the mothers.

When total amount of physical activity was used on behalf of strength of it, regression to HDL-C and Apo A-1 became slightly worse in the fathers, although there was no difference in significance level. In the children, however, borderline relationship disappeared. When only strength of physical activity and BMI were used as explanatory variable in the fathers, R² for HDL-C and Apo A-1 fell down to 0.10 (p < 0.01) and 0.05 (p < 0.05), respectively.

As shown by the standardized partial regression coefficient in Table 3, amount of alcohol habitually taken was significantly and most strongly of the four factors related to increase in both of HDL-C and Apo A-1 in the fathers. BMI was significantly related to decrease of both of them with a strength next to alcohol. Number of cigarette daily smoked was significantly related to decrease in HDL-C, but its inverse relationship to Apo A-1 was insignificant. Strength of physical activity did not have significant relationship with either of serum HDL-C or Apo A-1 concentration, although the coefficients had positive values. In the children, strength of physical activity had a significant relationship with increase of Apo A-1 and borderline relationship with HDL-C, but BMI did not have significant relationship with either of them.

In the fathers and children, in whom the multiple regression was significant or borderline, strength of the effects described above were compared among the four factors. In Table 4 are shown the increments of HDL-C (ΔHDL-C) and Apo A-1 (ΔApo A-1) and their 95 percent confidence intervals produced by changing the factor level from the mean of the bottom tertile to that of the top one as well as the ratio of the increments (ΔHDL-C/ΔApo A-1). This ratio, which expresses the HDL-C change accompanying a unit concentration change of Apo A-1, would be a reflection of average construction of HDL synthesized or catabolized under the effect of respective life style factors. HDL-C and Apo A-1 concentrations expected for each of tertile subgroups calculated based on the regression equations for fathers and children are shown in Figs. 1 and 2, respectively, together with the ratio of expected HDL-C to expected Apo A-1 concentrations.

In the fathers, ranges of concentration change of both HDL-C and Apo A-1 were greatest by variation of alcohol drinking level of all four factors (Figure 1). The ΔHDL-C and ΔApo A-1 expected by increase in alcohol drinking were about 1.5 and 2 times as large as the respective values expected by lowering BMI, and about 2 and 4 times as large as those expected by decreasing cigarette number in the children (Table 4). Resultant ratio of HDL-C to Apo A-1 became higher along with increase in physical activity and alcohol intake and along with decrease in BMI and cigarette number (Figure 1). However, increment of HDL-C concentration accompanying a unit increment of Apo A-1 (ΔHDL-C/ΔApo A-1 in Table 4) induced by alcohol drinking was less than that induced by the other factors, and was about three quarters as much as that induced by lowering BMI and about half of that in-
Table 1. Subject numbers and mean ± standard deviation of variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fathers (No. = 124)</th>
<th>Mothers (No. = 131)</th>
<th>Children (No. = 155)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of age</td>
<td>40.0 ± 3.0</td>
<td>38.0 ± 3.1</td>
<td>(10~11)</td>
</tr>
<tr>
<td>Strength of physical activity (kJ/kg-day)</td>
<td>25.1 ± 12.1</td>
<td>20.1 ± 9.2</td>
<td>44.4 ± 12.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>63.6 ± 8.4</td>
<td>52.2 ± 6.6</td>
<td>34.7 ± 6.0</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>167.0 ± 6.0</td>
<td>154.4 ± 4.9</td>
<td>141.9 ± 6.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7 ± 2.4</td>
<td>21.9 ± 2.6</td>
<td>17.1 ± 2.2</td>
</tr>
<tr>
<td>TC (mmol/liter)</td>
<td>4.69 ± 0.82</td>
<td>4.63 ± 0.71</td>
<td>4.41 ± 0.65</td>
</tr>
<tr>
<td>HDL-C (mmol/liter)</td>
<td>1.33 ± 0.33</td>
<td>1.47 ± 0.34</td>
<td>1.52 ± 0.34</td>
</tr>
<tr>
<td>Apo A-1 (μmol/liter)</td>
<td>54.2 ± 9.8</td>
<td>53.7 ± 8.5</td>
<td>53.4 ± 6.9</td>
</tr>
</tbody>
</table>

No.: Number of the subjects, kJ: Kilojoule, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, Apo A-1: Apolipoprotein A-1.

Table 2. Correlation coefficients of HDL-C and Apo A-1 in the 93 triplets of family members.

<table>
<thead>
<tr>
<th></th>
<th>Fathers vs children</th>
<th>Mothers vs children</th>
<th>Fathers vs mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>0.10</td>
<td>0.41***</td>
<td>-0.09</td>
</tr>
<tr>
<td>Apo A-1</td>
<td>0.19*</td>
<td>0.19*</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* p<0.10, ** p<0.01
HDL-C: High density lipoprotein cholesterol
Apo A-1: Apolipoprotein A-1

The increment of HDL-C concentration accompanying a unit increment of Apo A-1 (ΔHDL-C/ΔApo A-1 in Table 4) expected by strength of physical activity was greater than that expected by alcohol drinking in the fathers.

DISCUSSION

Total variation of HDL-C and Apo A-1 of the fathers were explicable as much as 29 and 23 percent, respectively, by strength of physical activity, obesity, and alcohol drinking and cigarette smoking habits in daily life. These values was higher than 18 percent reported by Fraser et al5), who investigated relationships to HDL-C in male adults by a follow-up study by adding fish consumption to the four factors similar to ours, and was also higher than 13 percent reported by...
Salonen et al\(^{20}\)), who included five more factors in the multiple regression model.

Our results from the fathers that alcohol drinking was related to high serum concentrations of HDL-C and Apo A-1 while cigarette smoking and obesity, to low ones were similar to the results by others\(^{8,9,11-13,17,19-22,26,27}\). The result that the effect of alcohol drinking was greatest was in agreement with that by Salonen et al.\(^{20}\) and Maeda et al.\(^{27}\), although physical exercise was not examined in the latter. In the report of Maeda et al.\(^{27}\), effects of alcohol and obesity on HDL-C were of similar strength, and this would have been due to a fact that heavy drinkers, with the daily amount of 80 g or more, in whom the effect was lesser were included.

Physical activity has been reported in several, but not in all, studies to be related to high concentrations of HDL-C and Apo A-1\(^{5,6,9,10,26,28,19}\). In our results strength of physical activity had not significant relationship with HDL-C and Apo A-1 in the parents, although the partial regression coefficient took positive values in the fathers. However, in the children, the relationship was significant or of borderline significance. Although high physical activity has been shown to increase HDL, Seals et al.\(^{30}\) showed strength of physical activity was related to the increase. In our study, strength of physical activity was greater in the children than in the parents as shown in Table 1, and this would have caused inconsistency of relationship of physical activity with HDL-C and Apo A-1 in the children and in the parents. Inconsistency of the effect of physical activity in various reports may be in part due to difference of the index used for physical activity.

In our study, these effects were obtained on the assumption that the level change of a factor did not affect the levels of the other factors. Such assumption would not be realistic, as increase in physical activity, for instance, is often accompanied by decrease in obesity. Therefore, the actual effect of each factor needs to be confirmed by a intervention study. According to one of such studies by Fraser\(^{9}\) who intervened the subjects by having them abstain from alcohol ingestion, the effect of exercise on serum HDL-C and Apo A-1 levels was much smaller than that by alcohol intake.

In addition to the quantitative difference, there seemed to be a qualitative one in the effects of the four factors in terms of distribution of HDL subfractions. The increment of HDL-C concentration per a unit increment of Apo A-1 concentration, shown by $\Delta$HDL-C/$\Delta$Apo A-1 in Table 4, induced by alcohol drinking was smaller than that expected by decrease in BMI or by abstaining from cigarette smoking. This finding is in agreement with that of Salonen et al.\(^{20}\) that alcohol was related to increase in both of HDL-C

![Figure 1](image-url)

**Figure 1.** Expected serum high density lipoprotein cholesterol (HDL-C) and apolipoprotein A-1 (Apo A-1) concentrations and their ratio of the fathers when each of strength of physical activity, body mass index (BMI), and alcohol drinking and smoking habits took the mean of the bottom (T1), the middle (T2) and the top (T3) tertile subgroups. Expected values were calculated through the multiple regression models shown in the Table 2.
and Apo A-1 while BMI and cigarette smoking were related only to HDL-C. It has been also reported that Apo A-1 concentration increased in low dose alcohol intake\(^1\) as well as in moderate dose\(^8,13\) and heavy drinkers\(^5\). However, HDL-C increase was not noted\(^9\) or lesser than that of Apo A-1\(^9\) in low dose drinkers. These and our findings would suggest that redistribution of HDL subfractions occurred so that a subfraction more rich in Apo A-1 increased relatively more than the one less rich by alcohol drinking in spite of apparent increase in resultant ratio of HDL-C to Apo A-1 shown in Figure 1.

### Table 4. Differential of high density lipoprotein cholesterol (ΔHDL-C) and that of apolipoprotein A-1 (ΔApo A-1) and their 95% confidence intervals* expected by varying the level of each variable from the mean of the bottom tertile to that of the top one and the ratio of the differentials.

<table>
<thead>
<tr>
<th>Variables</th>
<th>ΔHDL-C (mmol/liter)</th>
<th>ΔApo A-1 (μmol/liter)</th>
<th>ΔHDL-C/ΔApo A-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fathers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.08 [-0.03~ 0.19]</td>
<td>1.1 [-2.2~ 4.4]</td>
<td>72.7</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.20 [-0.30~ -0.09]</td>
<td>-4.4 [-7.8~ -1.1]</td>
<td>45.5</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.31 [0.19~ 0.42]</td>
<td>9.0 [5.6~ 12.4]</td>
<td>34.4</td>
</tr>
<tr>
<td>Cigarette</td>
<td>-0.16 [-0.27~ -0.05]</td>
<td>-2.4 [-5.8~ 0.9]</td>
<td>66.7</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.11 [-0.01~ 0.23]</td>
<td>2.4 [0.0~ 4.7]</td>
<td>45.8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.06 [-0.16~ -0.05]</td>
<td>-1.0 [-3.2~ 1.1]</td>
<td>60.0</td>
</tr>
</tbody>
</table>

* Shown in brackets below each of the differentials.

It has been shown that running and other physical activity elevated HDL\(_2\), an HDL subfraction less rich in Apo A-1, but lowered HDL\(_3\), a subfraction more rich in it, in men after ten weeks\(^10\) and one year\(^7\) or both of them\(^26\). In our results, significant relationship to Apo A-1 was obtained only for children, and ΔHDL-C/ΔApo A-1 due to physical activity in children tended to be greater than that due to alcohol in the fathers. From these and above findings it seems most likely that lowering BMI, abstaining from cigarettes, and probably increase in physical activity causes relative increase in an HDL subfraction less rich in Apo A-1 over the one with opposite quality than alcohol drinking does. Such inference was possible since both HDL-C and Apo A-1 levels were determined in the present study. However, since we did not directly determine serum levels of the HDL subfractions, this inference remains to be confirmed.

Recently, it has been suggested that HDL\(_2\) is more strongly related to coronary atherosclerosis than HDL\(_3\).\(^3\) Both protective effect of alcohol against ischemic heart disease\(^3)\) and doubtful result, especially, in moderate or higher drinking level\(^34\) are reported. According to Danielsson et al.\(^16\), qualitative change in HDL due to alcohol was individually different in terms which of HDL\(_2\) or HDL\(_3\) was elevated, and this finding suggests that alcohol would not
necessarily provide a protective effect against atherogenesis to those in whom HDL3 subfraction was elevated. Therefore, epidemiological studies that take HDL subfractional concentration into consideration is necessary to determine whether alcohol is truly protective through lipid metabolism. Unless an easy method to determine subfractional concentration is developed, HDL-C and Apo A-1 levels would be a useful proxy.

In the children, only several percent of total variation of HDL-C and Apo A-1 could be explained by strength of physical activity and obesity. Regression was even insignificant in the mothers. These results were compatible with the finding from the fathers that only 10 and 5 percent of total variation of HDL-C and Apo A-1, much smaller than that explained by the four factors, could be explained by combination of these two factors. HDL-C concentration of the children had a correlation with that of mothers with a coefficient of determination of 0.17, much higher than the coefficient in the regression by environmental factors, 0.03. This result may suggest that children of this age are affected more by a genetic factor than environmental factors. Although the correlation coefficient of HDL-C between mothers and children was higher than that of Apo A-1, the reason for this difference is beyond the scope of this paper and will be discussed in the following paper.

Bordeline relationship of BMI with HDL-C was obtained in the mothers in agreement with Haarbo et al.11 and others27 who showed that obesity was significantly related to decrease in HDL-C and Apo A-1 in post and premenopausal women. However, we could not obtain a significant relationship of physical activity to HDL-C and Apo A-1. One of the reasons for this negative finding would be due to a fact that the level of physical activity was lowest in the mothers and it might have been too low to affect them. Another reason would be shortness of subject number. Effect of nutrient intake such as carbohydrate on Apo A-1 has been reported35. So the factors such as dietary habit and sex hormones may have to be included in the analysis to find a better relationship in the mothers. Especially in children, effect of genetic heritability may have to be taken into consideration to obtain more significant relationship.

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