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原著：

Relation between Lifestyle Factors and High-Density Lipoprotein Cholesterol Subfraction Levels among Healthy Adults Living in a Rural District

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Serum levels of total high-density lipoprotein cholesterol (HDL-C) and its subfractions (HDL₂-C and HDL₃-C) were measured in 227 healthy Japanese adults. These values were related to a number of lifestyle factors.

On co-variance analysis, alcohol drinking had an independent contribution to HDL-C and HDL₃-C ($p < 0.05$) in males. Cigarette smoking showed no association with subfractions of HDL-C in males. Three physical activities—sport, work and leisure time activity—had no significant association with subfractions of HDL-C. The degree of obesity as expressed by body mass index (BMI) was significantly negatively related ($p < 0.05$) to HDL-C and HDL₂-C levels among females. Among males, although a trend test showed no statistically significant relation to BMI, HDL-C and HDL₂-C levels were significantly higher ($p < 0.05$) in the normal group ($20 \leq \text{BMI} < 25$) than in the obese group ($\text{BMI} \geq 25$).

①High-density lipoprotein subfractions ②Alcohol ③Obesity

INTRODUCTION

Serum high-density lipoprotein cholesterol (HDL-C) levels are associated with several lifestyle factors related to coronary heart disease (CHD) risk, such as alcohol drinking, cigarette smoking, lack of physical exercise and degree of obesity¹⁻⁴). However, the situation is more complex because high-density lipoprotein (HDL) is a heterogeneous mixture of lipoprotein particles that differ in lipid and apoprotein composition. DeLalla et al. first noted that HDL was heterogeneous by using analytical ultracentrifuge techniques⁵).

High-density lipoproteins can be separated into two major subclasses; HDL₂ (density: 1.063~1.125 g/ml) and HDL₃ (density: 1.125~1.21 g/ml). There is evidence that HDL₂ and HDL₃ differ in metabolism, and that HDL₂ contributes largely to the variability in total high-density lipoprotein⁶). Miller et al. showed that atherosclerosis as defined by coronary angiography is associated with lowered HDL₂-C rather than with HDL₃-C levels⁷). Bal-lantyne et al. showed that HDL₂-C, but not HDL₃-C, was reduced greater in survivors of myocardial infarction than in control subjects⁸). However, controversy still exists over the relative importance of

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HDL-C subfractions as predictors of CHD⁹⁾. The associations between these HDL subfractions and factors that affect total HDL are not yet well established. Therefore, we decided to investigate the relationship of HDL-C subfractions to lifestyle factors associated with coronary risk.

In Japan, few studies have examined the joint impact of lifestyle variables on cholesterol levels in the HDL-C subfractions¹⁰⁾. In the present study we examined the association between lifestyle factors and HDL-C subfraction levels in clinically healthy adults living in Yasu Town, northern Kyushu, Japan in 1991.

METHODS

Subjects

We used data from health examination surveys, and subjects under medical treatment for cardiovascular diseases were excluded, because lifestyle factors might have been affected by doctors' instructions. Then, the present analysis was restricted to the 65 males and 162 females who were between 30 and 79 years of age in 1991. In the occupational distribution of this study, farmers accounted for 71.4% and the full time farmers, 27.8%, of the subjects.

Lifestyle questionnaire

The questionnaire was self-administered and focused on alcohol drinking, cigarette smoking and physical activities. As for alcohol drinking, individuals were first asked whether they drink on average once a day. Those who drink every day were defined as drinkers, and they were further asked about the number of years they had been drinkers and the amount consumed per day. For analysis, alcohol drinking was divided into three groups on the basis of alcohol consumption. Smoking habits were classified using the reported number of cigarettes smoked per day. For analysis, current smokers were divided into two groups by the number of cigarettes per day. All ex-smokers who had stopped smoking more than six months before the beginning of this study were categorized as non-smokers. Questions on physical activity were based on the method of Baecke et al¹¹⁾. These concerned physical activity at work (work index), sport during

leisure time (sport index), and other physical activity during leisure time (leisure-time index). Body weight and height were measured with the subjects in light clothing and without shoes. The body mass index (BMI) was computed as weight in kilogram divided by height in meters squared.

Measurement of serum lipids.

Nonfasting blood was sampled from an antecubital vein using an evacuated tube. Time elapse since having breakfast was recorded for each subject. Using the data, we ascertained that there were no statistical differences in serum lipid levels with variation in the elapse of time. Serum was separated on the same day and transferred to a commercial laboratory (Creative Research Community, Fukuoka) for determination of HDL-C and HDL-C subfractions.

HDL-C was measured by a dextran sulfate-MgCl₂ method¹²⁾. HDL-C subfractions were separated with zonal ultracentrifugation using RPL42-T rotor (Hitachi Co. Ltd., Tokyo, Japan). The ultracentrifugation of lipoproteins was carried out according to the method used by Patsch¹³⁾. Subjects whose HDL-C subfraction recovery was below 85% or above 115% were excluded from the analysis¹⁴⁾. Statistical analysis

HDL-C levels and the levels of subfractions had a normal distribution. Co-variance analysis was used to assess the independent associations of lifestyle factors with HDL-C levels and its subfractions. Statistical computation was done by the Statistical Analysis System¹⁵⁾.

RESULTS

The mean values of age, concentrations of total cholesterol (TC), HDL-C and its subfractions are shown in Table 1 for both sexes. Males had a higher mean age than females. Mean TC, HDL-C and HDL₂-C were higher in females than in males, but these levels were not significantly different.

As shown in Table 2, 60% of males, but only 2% of females, were regular drinkers. Fifty-nine percent of males, but only 2% of females, were smokers. Physically active persons who usually participate in sports or exercise were 24.6% among males and the percentage was similar among

Table 1. Means and standard deviations (SD) of age and serum lipid levels by sex

Variables	Male (n=65)			Female (n=162)		
	Means	SD	Range	Means	SD	Range
Age (yrs)	59.0	13.0	31~ 79	55.0	13.7	30~ 78
T C (mg/dℓ)	197.4	38.9	102~313	203.5	37.6	115~333
HDL-C (mg/dℓ)	57.6	14.3	31~118	60.5	13.2	34~108
HDL ₂ -C (mg/dℓ)	37.3	12.8	6~ 77	41.6	14.0	6~ 84
HDL ₃ -C (mg/dℓ)	20.3	8.3	11~ 46	20.0	6.4	10~ 48

TC : Total cholesterol,

HDL-C : High-density lipoprotein cholesterol

Table 2. Distribution of lifestyle factors by sex

Lifestyle factors and their subgroups	Male		Female	
	n	%	n	%
Alcohol drinking				
Non-drinkers	16	24.6	129	79.6
Occasional drinkers	10	15.4	30	18.5
Regular drinkers	39	60.0	3	1.9
Cigarette smoking				
Non-smokers	20	30.8	159	89.1
Ex-smokers	7	10.7	0	0.0
Current Smokers	38	58.5	3	1.9
Physical exercise				
Inactive	49	75.4	121	74.7
Active	16	24.6	41	25.3
Degree of obesity (BMI)				
<25	48	73.8	127	78.4
≥25	17	26.2	35	21.6

females (25.3%). Obesity was found in 26.2% of males (BMI≥25) and 21.6% of females.

Tables 3 and 4 show independent relationships between the three HDL-C measurements and each variable after allowing for the effects of all the other variables.

For males, as shown in Table 3, the results indicated that both HDL-C and HDL₃-C had positive associations with alcohol drinking ($p<0.05$). Smoking was not associated with HDL-C nor its subfractions. A group of smokers (≤ 20 cigarettes per day) had the lowest levels of HDL-C and HDL₂-C compared with the other two groups, but the levels were not significantly different. HDL₃-C levels in

the heavy smokers group (>20 cigarettes per day) were lower than in the other two groups. For each of three physical-activity indices (sport, work and leisure-time activity), subjects were divided into two groups those above the mean value and those below. For leisure-time index, HDL-C and its subfraction levels in the high-score group were higher than those in the low-score group. However, for none of the three physical activity indices was there a statistically significant difference between the high-score group and the low-score group in the levels of HDL-C or its subfractions. Although BMI did not show a significant association with HDL subfractions on the trend test, the levels of both HDL-C and HDL₂-C in the normal group ($20 \leq \text{BMI} < 25$) were significantly higher than in the obese group ($\text{BMI} \geq 25$).

For females, as shown in Table 4, both alcohol drinking and cigarette smoking were excluded in this analysis, because there were few drinkers and few smokers. For both sport index and leisure-time index, the high-score groups showed higher levels of HDL-C and HDL₂-C than the low-score groups. But there was no significant difference. BMI showed a negative association with HDL-C and HDL₂-C ($p<0.05$).

DISCUSSION

DeLalla et al. found that females had significantly higher HDL₂-C levels than males, but similar HDL₃-C levels⁹. The higher HDL-C and HDL₂-C, but relatively similar HDL₃-C, levels in females as compared with males confirm other investigators' findings with a variety of different techniques^{16,17}.

In this study, we found almost the same sex

Table 3. Adjusted means of HDL subfractions according to categories of lifestyle factors among males

Categories	n	Adjusted means (SE) [#]		
		HDL-C(mg/dℓ)	HDL ₂ -C(mg/ℓ)	HDL ₃ -C(mg/dℓ)
Drinking (ml/day)				
None	16	49.7(4.1)	30.4(4.1)	20.7(2.6)
<54.6	46	57.2(2.7)	37.8(2.6)	18.3(1.7)
≥54.6	3	75.4(8.8)	38.9(8.2)	36.1(5.5)
		§		§
Smoking (cigarettes/day)				
None	27	61.3(3.8)	36.5(3.5)	25.0(2.4)
≤20	25	58.0(4.8)	31.7(4.5)	26.9(3.0)
>20	13	62.9(4.9)	38.9(4.5)	23.2(3.0)
Sport index				
<2.4	22	61.1(3.8)	35.6(3.5)	25.1(2.4)
≥2.4	43	60.4(4.3)	35.8(4.0)	25.0(2.7)
Work index				
<2.9	17	61.7(4.9)	36.2(4.5)	24.4(3.1)
≥2.9	48	59.7(3.4)	35.2(3.2)	25.6(2.1)
Leisure-time index				
<2.8	38	58.5(3.9)	33.2(3.7)	25.0(2.5)
≥2.8	27	63.0(4.0)	38.2(3.7)	25.0(2.5)
Body mass index				
<20	13	62.5(5.4)	38.4(5.0)	25.8(3.4)
20≤BMI<25	35	64.1(3.4)]*	38.8(3.2)]*	25.6(2.2)
≥25	17	55.6(4.5)]	30.0(4.2)]	23.7(2.8)

[#]: Based on analysis of co-variance including the lifestyle variables and age as independent variables.

SE: Standard error

*: p<0.05

§: Test for trend, p<0.05

Table 4. Adjusted means of HDL subfractions according to categories of lifestyle factors among females

Categories	n	Adjusted means (SE) [#]		
		HDL-C(mg/dℓ)	HDL ₂ -C(mg/dℓ)	HDL ₃ -C(mg/dℓ)
Sport index				
<2.4	60	58.6(1.9)	39.3(1.9)	20.3(0.9)
≥2.4	102	62.0(1.5)	43.3(1.5)	19.7(0.7)
Work index				
<2.9	83	60.5(1.7)	41.3(1.7)	20.2(0.8)
≥2.9	79	60.0(1.7)	41.3(1.7)	19.7(0.8)
Leisure-time index				
<2.8	79	58.9(1.7)	40.7(1.7)	19.1(0.8)
≥2.8	83	61.6(1.7)	41.9(1.7)	20.9(0.8)
Body mass index				
<20	27	64.8(2.6)	46.9(2.7)	18.8(1.3)
20≤BMI<25	100	60.1(1.3)	41.4(1.4)	19.8(0.7)
≥25	35	55.9(2.3)	35.6(2.4)	21.3(1.2)
		§	§	

Drinking and Smoking index were excluded because few females were drinkers or smokers.

[#]: Based on analysis of co-variance including the lifestyle variables and age as independent variables.

SE: Standard error

§: Test for trend, p<0.05

difference in the levels of HDL-C and its subfractions as in these previous reports.

An unusual feature in our study was the high proportion of obese subjects ($\text{BMI} \geq 25$), especially in males; it is clear that the distribution of obesity (male; 26%, female; 22%) is higher than the national level (14% and 18% respectively)¹⁸. We considered that this high level of obesity in males may be the result of a reduced burden of work (due to mechanization, automation or mortorization) and an abundance of food; and for many males some weight loss would be desirable. Three other important lifestyle factors—alcohol drinking, cigarette smoking, and lack of physical exercise—were shown to be similar to the national level¹⁸.

In the present study, co-variance analysis demonstrated that alcohol consumption was the major determinant of elevated HDL₃-C levels when allowance was made for other variables associated with alcohol such as cigarette smoking, physical activity and obesity. Previous studies have indicated that the major subfraction influenced by moderate alcohol intake is HDL₃-C, even when adjusted for other lifestyle variables^{17,19,20}. However, several abstracts indicate that both HDL₂-C and HDL₃-C rise in response to a moderate intake of alcohol in healthy men^{21,22}. The variation between different studies in findings on the relationship between HDL₂-C and alcohol may reflect a threshold effect, HDL₂-C levels only being affected at higher levels of intake. The mechanism by which alcohol affects HDL-C levels is unknown, but increased lipoprotein lipase production and decreased hepatic endothelial lipase levels have been suggested as contributing factors²³.

Smoking is a very important risk factor for ischemic heart disease²⁴. It has been thought that one reason for this is the effect of smoking on HDL-C, but mechanisms to explain the association remain obscure. Haffner et al. found HDL₃-C levels to be lower in smokers than in non-smokers, but observed no relationship between smoking and HDL₂-C¹⁷. On the other hand, Robinson et al. found no relation between cigarette smoking and HDL subfraction levels after allowing for confounding variables¹⁹. We also examined the cumulative

effects of smoking using the Brinkman Index, which takes into account the number of years of smoking. However, there was no relation between the Brinkman Index and HDL subfractions. Assessing the relation between smoking habits and HDL subfractions is made more difficult by the reported reduction since the 1950s of tar and nicotine in cigarette smoke²⁵.

Lakka et al. studied the association of physical activity with serum lipids in middle-aged men, controlling the major confounding factors²⁶. They found physical activity was associated consistently and independently with elevated serum HDL-C and HDL₂-C levels. However, there have been a few studies that found no significant association between degree of physical exercise and levels of HDL₂-C^{19,27}. In our present study, no relationship between physical activity and HDL subfractions was observed, although HDL₂-C levels among active subjects with high scores on leisure-time index were higher than low-score subjects for both sexes. The failure of our study to demonstrate a relationship between physical activity and HDL-C and its subfractions, particularly HDL₂-C, may be due to lack of intensity in the physical activities of the subjects. Lakka et al. suggest that there may be a threshold for an increase in HDL-C and HDL₂-C at a conditioning activity intensity of approximately 5-6 METs²⁶. Wood et al. reported that a one-year running program involving 8 or more miles per week over a year led to beneficial lipoprotein changes²⁸.

Several studies have shown an inverse relationship between HDL-C and obesity²⁹⁻³¹. This study confirms the findings of Albrink et al.³² and Haffner et al.¹⁷ that BMI has a negative association with HDL₂-C, particularly in women, but no apparent relationship with HDL₃-C. One recent study has demonstrated that high HDL levels are associated with high levels of lipoprotein lipase³³. In obesity, diminished lipoprotein lipase activity could reduce the rate of very low-density lipoprotein degradation, resulting in a diminished HDL formation, particularly for the HDL₂ subfraction.

The findings of our study are generally in agreement with previous observations. Our results

indicate the need for people in the district to modify their lifestyle, and in particular, for obese males to reduce their weight to prevent cardiovascular disease.

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農村地区における健康成人の生活習慣と HDL-C の主要成分 (HDL₂-C, HDL₃-C) との関連

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HDL-C の主要分画が冠状動脈性心疾患 (CHD) の予測者になりえるかについては、未だ論議が続いている。これらの分画が、CHD のリスクと関係のある生活習慣 (飲酒、喫煙、身体活動、肥満度) と関連するかについても明確でない。そこで本研究は、健康成人 227 名 (30 歳～79 歳) を対象に、HDL 分画に対する各生活習慣の独立した関連を検討するため、共分散分析法を用いて傾向検定 (test for trend) を行った。ただし、女の飲酒と喫煙はサンプル数が少なく、解析から除外した。その結果、男では飲酒が HDL-C, HDL₃-C との関連を示したが、喫煙は両分画ともに関連を示さなかった。身体活動はスポーツ、仕事、余暇活動に分けて検討したが、男女ともに有意な関連はみられなかった。肥満度は HDL-C, HDL₂-C との間の負の関連が、女にみられた。男では傾向検定では有意な関連を示さなかったものの、肥満群 (Body Mass Index ≥ 25) の HDL-C, HDL₂-C が、正常群 ($20 \leq \text{BMI} < 25$) に比べ、有意に低い値を示した。

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