ORIGINAL CONTRIBUTION

Relative Contributions of Selected Genetic and Lifestyle Factors to Inter-Individual Variations in Serum Lipid and Apolipoprotein Levels

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Serum lipid and apolipoprotein profiles are influenced by both genetic and environmental factors. However, the relative effects of these factors on the phenotypic variations remain unclear. In this study, the relative contributions of selected genetic and lifestyle factors to inter-individual variations in serum lipid and apolipoprotein levels were estimated by using the multiple regression model in a rural Japanese population. Four restriction fragment length polymorphisms (RFLPs) were tested with Xba I and EcoR I at the apolipoprotein B (apo B), and Msp I and Sac I at the apo AI-CIII gene loci. The contribution of individual RFLP to serum total cholesterol ranged from 0.08% to 1.60%; serum low density lipoprotein cholesterol, 0.06% to 1.69%; triglycerides, 0.04% to 0.89%; apo B, 0.06% to 1.99%; high density lipoprotein cholesterol, 0.05% to 2.59%; apo AI, 0.54% to 2.73%; apo AII, 0.12% to 1.96% and apo CIII, 0.13% to 0.48%. These percentages were almost the same as or a little lower than those of some lifestyle variables—dietary factors (Keys dietary score, and energy-adjusted intake of carbohydrate, fiber and n-3 fatty acids), smoking, alcohol consumption and physical activity—to the serum traits. J Epidemiol, 1995; 5: 187-196.

The level of serum lipid or apolipoprotein at any given time is a result of interactions of genetic endowment with lifestyle over the entire lifespan. For the serum lipid levels, however, the relative contributions of genetic and lifestyle factors are not clear; it is very difficult to arrive at an assignment of weights.

The aim of the present study is to quantitatively estimate the relative contributions of selected genetic and lifestyle factors to the inter-individual variations in serum lipid and apolipoprotein levels in a rural Japanese population, using the multiple regression model. Serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), apolipoprotein B (apo B), high density lipoprotein cholesterol (HDL-C), apo AI, apo AII and apo CIII levels were determined as the dependent variables of the function. On the basis of established or putative roles of the candidate gene products in lipoprotein metabolism, four restriction fragment length polymorphisms (RFLPs) were selected with Xba I and EcoR I at the apo B, and Msp I and Sac I at the apo AI-CIII gene loci as the genetic independent variables. As for lifestyle factors, dietary intake, cigarette smoking, alcohol consumption and physical activity were also entered into the model for the current study.

SUBJECTS AND METHODS

Study Area

The study area is H-Y district, Shiso County, Hyogo Prefecture, Japan. This district is an agricultural area where rice and vegetables are produced on a relatively small scale. However, majority of the residents are not only farmers but also regular employees of offices or factories. They are engaged in farming only during the rice-planting and harvesting seasons.

Subjects

All residents aged 40 to 69 years of H-Y district, 1,721...
men and 1,645 women, were invited to undergo an examination in 1992, which was conducted under a Japanese law for the prevention of cardiovascular disease. Of them, 61% men and 64% women responded to the invitation. From the respondents, 105 men and 105 women were selected at random. Individuals who refused to be genotyped, who took lipid-lowering medication, and who suffered from diabetes mellitus, chronic renal and hepatic diseases and chronic alcohol abuse were excluded. Finally, 80 men and 95 women were recruited for the study.

We obtained informed consent from all of the subjects. Ethical approval for the present study was granted by the Ethical Review Committee, Medical Research Institute, Tokyo Medical and Dental University.

**Measurement of serum lipids and apolipoproteins**

Venous blood was drawn in overnight fasting state. Serum TC and TG were determined by enzymatic methods, and serum HDL-C by dextran sulfate/Mg²⁺ precipitation. The level of serum LDL-C was calculated by using the Friedewald's formula. As to the control over accuracy and precision of serum lipid measurement, the standardization was achieved by participation in the CDC Lipid Standardization Program through Osaka Prefectural Center for Adult Diseases, Japan.

Serum apo B, apo AI, apo All and apo CIII concentrations were measured by the method of turbidimetric immunoassay (TIA).

**DNA analysis**

The subjects were genotyped for four RFLPs with Xba I and EcoRI at the apo B, Msp I and Sac I at the apo AI-CIII gene loci, using the established methods.

Different 20 samples were measured twice for each of Xba I, EcoRI, Sac I and Msp I polymorphisms to confirm the reproducibility, which was found to be 100%.

**Assessment of lifestyle factors**

Standardized questionnaire methods were used to assess lifestyle factors by trained interviewers or dietitians.

Since there was no ex-smoker, the subjects were classified into three groups: 1) non-smokers, 2) smokers≤20 cigarettes/day, and 3) smokers>20 cigarettes/day. Alcohol-drinking habits included the principal forms of drinking—beer, wine, whisky, sake and shou chu—and the amount of drinking. The daily amount of alcohol consumption was calculated in grams according to the ethanol content in a serving of each type of alcoholic beverage and then converted into the number of drinks per day (1 drink ≈ 12 grams of ethanol). Alcohol consumption was categorized as 1) non-drinkers, 2) drinkers≤2 drinks/day and 3) drinkers>2 drinks/day.

A 24-hour recall method was employed to assess the dietary intake. Subjects were helped to estimate the quantities of food consumed by use of wax food models, full-sized photographs of foods, standardized portion size scales, household measures, serving dishes and rulers. Nutrient intakes were calculated using a computer database of the Japanese Standard Tables of Food Composition, the 4th Revision, 1982.

“Energy-adjusted” nutrient intakes were computed by the residual method of Willett and Stampfer, and classified into four categories by quartile.

According to the classic 'diet-heart' hypothesis, high intake of saturated fats and cholesterol or low intake of polyunsaturated fats increase the levels of serum TC or LDL-C. Increased intake of dietary fiber can exert a beneficial effect on blood lipids. N-3 fatty acids, which are predominately provided by fish that is highly consumed by the Japanese (90 grams/day in H-Y district), have a variety of favorably physiologic effects on serum HDL-C, including a potent reduction in LDL-C and TG. In addition, Japanese diets are characterized by high carbohydrate, the main source of which is steamed rice, in contrast to Western diets with high animal fat intake. Thus, Keys dietary score (a combination of saturated fatty acids, polyunsaturated fatty acids, cholesterol and energy intake), fiber, n-3 fatty acids and carbohydrate were employed as the dietary factors for the analyses.

Individual habits of physical activity were assessed by a standardized questionnaire which included information on the principal energy-consuming tasks and the time spent in doing them. Average daily energy expenditure during the preceding year was calculated by the method of Japanese Ministry of Health and Welfare, and categorized into three groups: 1) light, 2) moderate and 3) heavy.

**Statistical analysis**

All statistical analyses were made by using SAS software, Ver. 6.08.

Since the values for serum TG and apo CIII were not normally distributed, they were logarithmically transformed. The transformed values were used for statistical analyses.

ANOVA was performed using the general linear model procedure (PROC GLM) to estimate the association of each gene polymorphism with individual serum trait and to obtain the least-square mean by genotype class. Sex and age were included in the analysis to adjust for their effects on serum lipoproteins. Since subjects homozygous for the presence of the Xba I site (X+X+) and the absence of the EcoRI site (E−E−) were very few in H-Y district, they were combined with those heterozygous for the corresponding alleles.

The relative contributions of selected genetic and lifestyle factors to the inter-individual variations in serum lipid and apolipoprotein levels were estimated by using the multiple regression analysis. Dependent variables were
Table 1. Genotype-specific levels [(mean ± SE) or median (95% CI)] of serum lipids and apolipoproteins, adjusted for sex and age.

| Xba I | No. | TC* (mmol/L) | LDL-C* (mmol/L) | TG** | APO B* (mg/dL) | HDL-C* (mmol/L) | APO AI* (mg/dL) | APO AI** (mg/dL) | APO CII*** (mg/dL) |
|-------|-----|--------------|-----------------|------|----------------|----------------|----------------|----------------|------------------|
|       | -/- | 158          | 5.24±0.08       | 3.28±0.07 | 2.25 (2.06–2.47) | 98.75±2.07     | 1.46±0.03 | 160.05±2.20 | 36.39±0.62 | 12.30 (11.75–12.87) |
|       | +/- | 17           | 5.40±0.25       | 3.40±0.22 | 2.71 (2.16–3.39) | 104.86±6.55     | 1.28±0.08 | 151.73±6.37 | 36.07±1.98 | 12.59 (10.51–15.08) |
| p     | NS  | NS           | NS              | NS   | NS             | NS             | <0.05       | NS             | NS               |
| EcoR I|       |              |                 |      |                |                |             |                |                  |
|       | +/- | 24           | 5.02±0.20       | 3.17±0.18 | 2.30 (1.92–2.76) | 92.18±4.89     | 1.35±0.07 | 153.43±5.65 | 33.06±1.60 | 12.30 (10.74–14.09) |
| p     | NS  | NS           | NS              | NS   | NS             | NS             | <0.05       | NS             | NS               |
| Sac I |       |              |                 |      |                |                |             |                |                  |
|       | -/- | 71           | 5.41±0.12       | 3.41±0.11 | 2.20 (1.92–2.52) | 102.41±3.10     | 1.51±0.04 | 169.90±3.22 | 38.07±0.92 | 12.02 (10.98–13.16) |
|       | +/+ | 87           | 5.14±0.10       | 3.21±0.10 | 2.36 (2.15–2.58) | 97.65±2.80     | 1.41±0.04 | 160.94±2.91 | 35.71±0.83 | 12.30 (11.24–13.46) |
| p     | NS  | NS           | NS              | NS   | NS             | NS             | <0.05       | NS             | NS               |
| Msp I |       |              |                 |      |                |                |             |                |                  |
|       | -/- | 39           | 5.17±0.16       | 3.35±0.15 | 2.47 (2.06–2.96) | 100.60±4.23     | 1.37±0.05 | 160.97±4.47 | 33.28±1.26 | 12.88 (11.77–14.10) |
|       | +/+ | 83           | 5.16±0.11       | 3.24±0.10 | 2.25 (2.06–2.47) | 95.72±2.86     | 1.43±0.04 | 162.30±3.01 | 34.81±0.85 | 12.02 (10.98–13.16) |
| p     | NS  | NS           | NS              | NS   | NS             | NS             | <0.05       | NS             | NS               |

*TC=total cholesterol; LDL-C=low density lipoprotein cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; APO=apolipoprotein.

**Logarithmically transformed values were used for analyses and medians (95% confidence interval) were presented.

***Differences of levels among 2 or 3 genotypes were tested by analysis of variance controlling for sex and age.

RESULTS

Associations between RFLPs and serum lipids or apolipoproteins

The results of the ANOVAs are shown in Table 1. Statistically significant associations were found between Xba I genotype and serum HDL-C and apo A1; EcoR I genotype and serum apo B and apo AI; Sac I genotype and serum HDL-C, apo AI and apo AII; and Msp I genotype and serum apo AI and apo AII.

Least-square means for each significant genotype-phenotype association suggested that the genotypes had an approximately linear effect on the serum lipoproteins (a gene-dosage effect). This might mean that the effect of the heterozygosity was intermediate to the effects of the two homozygosity for Sac I and Msp I RFLPs.

Associations between lifestyle factors and serum lipids or apolipoproteins

The same ANOVAs were done to estimate the associations between lifestyle factors and serum biochemical traits (Appendix 1). Our findings were almost consistent with the existing knowledge. As for dietary intakes, significant associations were observed between carbohydrate intake and serum apo B and HDL-C; dietary fiber and serum LDL-C and HDL-C; and Keys dietary score and serum LDL-C. Smoking was significantly associated with serum LDL-C, log TG and HDL-C. The positive association of drinking or physical activity with serum HDL-C, apo AI and apo AII was statistically significant and relatively strong.

Multiple regression analysis

Multiple regression analysis was performed to estimate the relative contributions of selected genetic and lifestyle factors to the inter-individual variations in serum lipid or apolipoprotein levels. These results are shown in Table 2 and Figure 1. The model included sex, age, four genetic polymorphisms, four dietary factors, smoking, drinking and physical activity (13 variables in total) as the independent variables and accounted for 6.98%, 13.17%, 6.99%, 13.43%, 22.54%, 20.17%, 16.55%, and 8.75% ($R^2$) of the total variation in serum TC, LDL-C, log TG, apo B, HDL-C,
### Table 2. Summary of multiple regression analyses

| Independent | TC (Std. $\beta$, $P \times 100$) | LDL-C (Std. $\beta$, $P \times 100$) | TG (Std. $\beta$, $P \times 100$) | APO B (Std. $\beta$, $P \times 100$) | HDL-C (Std. $\beta$, $P \times 100$) | APO AI (Std. $\beta$, $P \times 100$) | APO All (Std. $\beta$, $P \times 100$) | APO CII (Std. $\beta$, $P \times 100$) |
|-------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Xba I       | 0.032 0.084                    | 0.031 0.085                    | 0.100 0.886                    | -0.170 2.591*** | -0.125 1.395**            | -0.066 0.397                    | 0.049 0.214                    |                                 |
| EcoR I      | 0.072 0.464                    | 0.026 0.662                    | 0.022 0.044                    | 0.160 1.988**            | 0.088 0.686                  | 0.116 0.958                    | 0.149 1.964***            | 0.031 0.085                  |
| Sac I       | -0.213 1.597*                  | -0.199 1.690**                 | 0.004 0.043                    | -0.129 0.585             | -0.162 2.212***            | -0.268 2.725***                 | -0.191 1.668**            | -0.060 0.125                 |
| Msp I       | -0.068 0.170                   | -0.079 0.233                   | -0.051 0.144                   | -0.040 0.060             | -0.038 0.054                | 0.115 0.536                    | 0.058 0.124                  | -0.114 0.481                 |
| carbohydrate| 0.341 0.092                    | 0.600 0.200                    | 0.039 0.087                    | 0.147 1.700**             | -0.166 2.281***            | -0.114 0.827                   | -0.043 0.106                 | 0.217 2.025**                |
| fiber       | -0.146 1.420*                  | 0.154 1.670**                  | 0.154 2.134**                  | -0.162 2.184**           | -0.136 1.347*               | -0.053 0.233                   | -0.099 0.817                 | -0.070 0.300                 |
| n-3 fatty acids | -0.099 0.685                | -0.150 1.450**                 | -0.087 0.529                   | -0.150 1.797**           | 0.161 2.202**               | 0.031 0.058                    | 0.028 0.048                  | -0.070 0.295                 |
| Keys score  | 0.149 1.528*                   | 0.254 2.673***                 | -0.028 0.054                   | 0.056 0.224              | 0.044 0.140                 | 0.054 0.225                    | 0.022 0.034                  | 0.055 0.218                  |
| smoking     | -0.039 0.090                   | -0.138 1.358*                  | 0.208 2.603***                 | 0.159 1.980**            | -0.137 1.348*               | 0.058 0.199                    | 0.085 0.431                  | 0.153 1.405*                 |
| drinking    | -0.017 0.062                   | -0.150 1.528*                  | 0.046 0.113                    | -0.041 0.076             | 0.263 3.149****            | 0.315 4.525****                 | 0.363 5.985****            | 0.278 2.963****             |
| PA*         | 0.058 0.238                    | 0.033 0.077                    | -0.049 0.123                   | 0.030 0.067              | 0.137 1.347*               | 0.251 4.481****                 | 0.243 4.264****            | 0.039 0.108                 |
| sex         | -0.037 0.047                   | -0.132 0.765                   | 0.017 0.010                    | -0.060 0.121             | 0.208 2.244***            | 0.292 2.897***                   | 0.105 0.374                 | 0.049 0.082                 |
| age         | -0.078 0.507                   | -0.139 1.375*                  | -0.062 0.216                   | -0.161 2.141**           | 0.188 2.935***            | 0.116 1.115*                    | -0.064 0.337                | -0.073 0.444                 |

***p<0.001; **0.01≤p<0.05; *0.05≤p<0.01; *0.10≤p<0.15

*TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides;
HDL-C = high density lipoprotein cholesterol; APO = apolipoprotein.

*Logarithmically transformed values were used for analyses.

*Standardized regression coefficient

*PA = physical activity.
apo AI, apo AII and log apoCIII, respectively (Figure 1). The contribution, $P_i$, of individual RFLP to inter-individual variation in serum TC was 0.08% to 1.60%, 0.06% to 1.69% in LDL-C, 0.04% to 0.89% in log TG, 0.06% to 1.99% in apo B, 0.05% to 2.59% in HDL-C, 0.54% to 2.73% in apo AI, 0.12% to 1.96% in apo AII and 0.09% to 0.48% in log apoCIII. In general, $P_i$ for a RFLP did not appear to be larger than that for a lifestyle factor within the model: 2.67% for Keys dietary score (largest among the lifestyle factors selected) and 1.69% for Sac I (largest among the RFLPs tested) in LDL-C, 2.60% for smoking and 0.89% for Xba I in log TG, 2.18% for dietary fiber and 1.99% for EcoR I in apo B, 3.15% for drinking and 2.59% for Xba I in HDL-C, 4.53% and 4.48% respectively for drinking and physical activity and 2.73% for Sac I in apo AI, 5.99% and 4.26% respectively for drinking and physical activity and 1.96% for EcoR I in apo AII, and 2.96% for drinking and 0.48% for Msp I in log apoCIII, although $P_i$ for Sac I (1.60%) was almost as large as that for Keys dietary score (1.53%) in TC.

### Allele frequencies and serum lipid levels

The allele frequencies of four RFLPs for apo B and apo AI-CIII genes are shown in Appendix 2. The minor allele frequencies of Xba I and EcoR I RFLPs in H-Y district were much lower than those in Caucasian populations\(^\text{12,13}\), although they were almost the same as those from other Japanese\(^\text{14}\) and Chinese populations\(^\text{15}\).

On the other hand, the minor allele frequencies of Sac I and Msp I RFLPs were four times or more than those in Caucasian populations\(^\text{13}\), but they were not different from those in other Japanese populations\(^\text{16-18}\).

There was no significant deviation of observed genotype frequencies from those predicted by the Hardy-Weinberg law in H-Y district (data not shown).

The means and standard deviations of serum lipids and apolipoproteins are presented in Appendix 3. The levels of serum TC in H-Y district were similar to those of the whole Japan\(^\text{19}\), although the average values of serum HDL-C were a little lower. Therefore, the subjects of the present study did not appear to differ markedly from average Japanese.
DISCUSSION

Associations between RFLPs and serum lipids or apolipoproteins

Genotypes of Xba I RFLP at the apo B gene locus were not associated with any apo B-related traits in H-Y district (Table 1). The lack of the significant association was also observed in another Japanese population44, while the significant associations were observed in several Caucasian populations20-24. Xba I polymorphism results from a silent C to T transversion in the 26th exon of the apo B gene and does not change the apo B amino acid sequence25. Therefore, this polymorphism might work as a linkage marker for the particular traits in some populations but not in others. On the other hand, Xba I genotypes were related to serum HDL-C and apo AI. Our findings agreed with those from Asian studies15,26. Although the basis for these associations remains unknown, the allelic variant may be in linkage disequilibrium with another functional mutation within the candidate gene or even at another gene that is physically or genetically linked to the gene tested26. In this case, the variant serves as a marker for the actual causative mutation.

Genotypes of EcoR I at the apo B locus were significantly associated with serum apo B. This association may suggest that the genetic variants which encode structural changes in the protein might directly or indirectly affect the lipoprotein metabolism and serum apo B concentration27. However, the positive, negative and non-associations took nearly equal share in the number of the literatures cited15,20,22,28-30). Hegele et al. reported that the lack of a significant genotype-phenotype association for EcoR I was not surprising, because functional studies failed to show that this protein polymorphism had an effect31. On the other hand, a significant association of EcoR I with serum apo AII might be explained by the linkage disequilibrium hypothesis.

Genotypes of Sac I and Msp I RFLPs at the apo AI-CIII locus were shown to be associated with variations in serum HDL-C, apo AI and apo AII. Particularly, the significant association of Sac I polymorphism with serum HDL-C is consistent with many other studies31-33. This association could be due to linkage disequilibrium between this site polymorphism and functional variants in the promoter region of apo CIII gene44. However, some other studies showed no association of Sac I polymorphism with HDL-C25,26. The persons with M+M+ genotype of Msp I RFLP tended to have higher level of serum HDL-C37 or apo AI32 than those with M−M− genotype.

Relative contributions of genetic and lifestyle factors to serum lipids and apolipoproteins

In the present study, the contribution, Pi, of individual RFLP to inter-individual variation in serum TC, LDL-C, log TG, apo B, HDL-C, apo AI, apo AII and log apoCIII was between 0.04% and 2.73% (Table 2).

In a study in a genetically isolated population31, the multiple regression analysis was performed to estimate the percentage of phenotypic variation determined by genetic variation. The model included sex, age, log BMI, colony of origin and 4 to 8 genotypes as the independent variables. All of the candidate genes accounted for 7.8% of the total variation in plasma TC, 7.6% of LDL-C, 7.3% of non-HDL-C, 7.2% of apo B, 3.2% of log TG and 5.1% of HDL-C. The partial R² (presented as Pi in our study) of individual RFLP to serum trait ranged from 0.35% to 2.96%. Peacock et al.22 reported that EcoR I genotypes explained 1.3%, 1.6%, 2.9%, 2.2%, 0.5% and 3.0%, respectively, of total variations in serum TC, LDL-C, HDL-C, TG, apoAI and apoB in Swedish healthy individuals. Kesling et al.38 reported that the contribution of Xba I RFLP to serum LDL-C was 1.39%; HDL-C, 0.40%; apo AI, 0.11%; and EcoR I to apo AI, 2.99% (all adjusted for 6 covariates).

In the present study, the contribution of a lifestyle factor tended to be a little larger than that of any RFLP in the corresponding model. Pi of individual dietary factor ranged from 0.03% to 2.67%, smoking from 0.09% to 2.60%, drinking from 0.06% to 5.99%, and physical activity from 0.07% to 4.48% (Table 2, Figure 1). According to a study by Perusse et al.39, diet, physical activity, smoking and drinking accounted for 6% of the total variation in serum LDL-C and 13% in serum HDL-C. These percentages are close to our data: 8.96% in LDL-C and 11.81% in HDL-C. Freeman and colleagues40 observed that the contribution of drinking, smoking and age to the level of HDL-C was 10.2% (7.4% in our study). Adachi et al.41 reported that the proportion of drinking, smoking and dietary variables was 16.9% for apo B, 37.7% for apo AI and 35.0% for apo CIII. The corresponding figures in the present study were 8.0%, 6.0%, and 7.2%.

Several family and twin studies mainly in Caucasian populations reported the genetic proportions ranged from 40% to 68% for serum LDL-C39,42,43, 14-66% for apo B44,45, 36-62% for HDL-C39,44,47, and 0-53% for apo AI44,45,46. These figures were much higher than our and other candidate gene studies, although the methods are not comparable with each other. These differences might be, at least in part, due to their overestimation of the genetic part of the inter-individual variations in serum lipids or apolipoproteins besides ethnic difference. First, the overestimation might come from “shared environment”. Family members were living together at a home for a long time, and twins grew up together at least in their early life. Second, “family aggregation” of serum lipids and lipoproteins in families might result in the overestimation also49. Third, a study50 reported that the contribution of
It should be also stressed to add more genetic markers to the model and/or to combine them, e.g., to investigate the haplotypes, because the multiple common mutations have more important effect on the "normal" inter-individual variations in serum lipids.

In the present study, it is the limitation that 24-hour recall method was used for assessing the dietary intake, because this measure does not reflect long-term dietary habits retrospectively. To overcome this weak point more or less, the absolute value of dietary intake was not employed but categorized into four patterns by quartile.

Obesity-related indices were not included in the analyses, because they were related not only to the energy output and input but also to many host factors, i.e., because it is difficult to say clearly if they are lifestyle markers or genetic markers.

In conclusion, some polymorphisms in selected candidate genes appeared to be associated with some serum lipids or apolipoproteins, but the effect of individual RFLP on the inter-individual variations in serum lipids was relatively subtle. There was no marked difference between the contribution of individual RFLP and that of individual lifestyle factor to any serum trait in the present study. We did not find out any convincing evidence that the role of the genetic factors was more important than that of the environmental factors in lipoprotein metabolism in our study population, although many researchers reported that the environmental factors are less important than the genetic factors in Western populations[12].

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### Appendix 1. Associations between lifestyle factors and serum lipids and apolipoproteins, adjusted for sex and age

| Diet | Level of Independent | TC* (mmol/L) | LDL-C* (mmol/L) | TG** (mmol/L) | APO B* (mg/dL) | HDL-C* (mmol/L) | APO A* (mg/dL) | APO AII* (mg/dL) | APO CIII* (mg/dL) |
|------|---------------------|-------------|----------------|--------------|---------------|----------------|---------------|----------------|--------------------|
| Carbohydrate | 1 | 5.16±0.15 | 3.25±0.14 | 2.15(1.88–2.46) | 94.66±3.95 | 1.5±0.05 | 163.84±4.20 | 35.78±1.20 | 12.02(10.96–13.18) |
| | 2 | 5.13±0.15 | 3.19±0.13 | 2.20(1.92–2.52) | 95.81±3.90 | 1.4±0.05 | 161.81±4.15 | 35.81±1.19 | 12.02(10.96–13.18) |
| | 3 | 5.44±0.15 | 3.37±0.13 | 2.59(2.26–2.96) | 102.35±3.90 | 1.4±0.05 | 170.28±4.16 | 37.24±1.19 | 13.18(10.02–14.45) |
| | 4 | 5.19±0.15 | 3.31±0.13 | 2.25(1.97–2.58) | 110.34±3.96 | 1.3±0.05 | 157.06±4.21 | 36.61±1.21 | 13.49(12.33–14.76) |
| Fiber | 1 | 5.67±0.15 | 3.68±0.14 | 2.10(1.84–2.41) | 106.11±3.91 | 1.6±0.05 | 166.32±4.20 | 37.19±1.19 | 11.22(10.25–12.28) |
| | 2 | 5.45±0.15 | 3.59±0.13 | 2.30(2.01–2.64) | 99.34±3.91 | 1.4±0.05 | 163.01±4.38 | 37.06±1.19 | 12.02(10.96–13.18) |
| | 3 | 5.34±0.15 | 3.21±0.14 | 2.20(1.92–2.52) | 95.57±3.42 | 1.4±0.05 | 163.63±4.20 | 35.48±1.24 | 12.02(10.96–13.18) |
| | 4 | 5.30±0.15 | 3.18±0.13 | 2.59(2.26–2.96) | 96.06±4.07 | 1.3±0.05 | 160.08±4.33 | 35.66±1.22 | 13.49(12.33–14.76) |
| n-3 Fatty Acids | 1 | 5.41±0.14 | 3.69±0.13 | 2.47(2.16–2.83) | 109.78±3.82 | 1.3±0.05 | 165.05±4.37 | 37.38±1.23 | 12.02(10.96–13.18) |
| | 2 | 5.12±0.14 | 3.42±0.13 | 2.53(2.21–2.89) | 99.91±3.98 | 1.4±0.05 | 163.32±4.20 | 37.19±1.18 | 13.49(12.33–14.76) |
| | 3 | 5.10±0.15 | 3.11±0.13 | 2.05(1.79–2.35) | 93.90±3.84 | 1.4±0.05 | 159.50±4.28 | 34.79±1.21 | 11.48(10.49–12.57) |
| | 4 | 5.09±0.14 | 3.08±0.13 | 2.20(1.92–2.52) | 93.53±3.90 | 1.5±0.05 | 165.22±4.21 | 36.07±1.19 | 11.75(10.73–12.86) |
| Keys Score | 1 | 5.15±0.15 | 3.11±0.14 | 2.59(2.26–2.96) | 103.43±4.05 | 1.3±0.05 | 163.26±4.36 | 37.27±1.23 | 12.88(11.77–14.10) |
| | 2 | 5.19±0.15 | 3.18±0.13 | 2.20(1.92–2.52) | 95.81±3.91 | 1.4±0.05 | 161.16±4.21 | 36.61±1.19 | 11.75(10.73–12.86) |
| | 3 | 5.24±0.15 | 3.38±0.13 | 2.15(1.88–2.46) | 95.19±3.92 | 1.4±0.05 | 164.36±4.22 | 35.10±1.19 | 11.75(10.73–12.86) |
| | 4 | 5.49±0.15 | 3.68±0.13 | 2.30(2.01–2.64) | 103.00±3.94 | 1.4±0.05 | 164.35±4.24 | 36.51±1.20 | 12.59(11.50–13.78) |
| Drinking | 1 | 5.22±0.12 | 3.35±0.11 | 2.30(2.01–2.64) | 99.83±3.11 | 1.3±0.04 | 154.47±3.16 | 33.86±0.88 | 11.75(10.73–12.86) |
| Non-drinker | 2 | 5.35±0.17 | 3.42±0.16 | 2.20(1.84–2.64) | 99.76±4.60 | 1.4±0.06 | 164.69±4.70 | 36.06±1.31 | 12.30(10.74–14.09) |
| | 3 | 5.17±0.19 | 3.03±0.17 | 2.36(1.97–2.83) | 98.98±5.03 | 1.5±0.06 | 182.37±5.10 | 42.56±1.43 | 13.80(12.06–15.81) |
| | 4 | 5.46±0.15 | 3.55±0.14 | 2.30(2.01–2.64) | 103.00±3.94 | 1.4±0.05 | 164.35±4.24 | 36.51±1.20 | 12.59(11.50–13.78) |
| Smoking | 1 | 5.45±0.09 | 3.56±0.09 | 2.15(1.97–2.35) | 97.46±2.49 | 1.5±0.03 | 162.20±2.68 | 35.96±0.76 | 12.02(11.49–12.58) |
| Non-smoker | 2 | 5.26±0.26 | 3.31±0.24 | 2.10(1.60–2.76) | 94.83±6.90 | 1.5±0.09 | 168.91±7.44 | 36.66±2.11 | 11.75(9.81–14.07) |
| | 3 | 5.18±0.20 | 3.11±0.18 | 2.90(2.42–3.48) | 109.14±5.20 | 1.3±0.07 | 164.05±5.60 | 37.79±1.59 | 13.80(12.06–15.81) |
| | 4 | 5.50±0.12 | 3.28±0.11 | 2.30(2.01–2.64) | 101.19±3.12 | 1.5±0.04 | 172.64±3.16 | 39.01±0.89 | 12.59(11.50–13.78) |

a: TC=total cholesterol; LDL-C=low density lipoprotein cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; APO=apolipoprotein.
b: Logarithmically transformed values were used for analyses and medians (95% confidence interval) were presented.
c: Dietary independent were categorized by quartile.
d: Differences of levels were tested by analysis of variance controlling for sex and age.
Appendix 2. Allele frequencies of RFLPs, and lifestyle factors.

| Allele Frequencies (%) | Men     | Women   |
|------------------------|---------|---------|
| Xba I (++)             | 0.069   | 0.047   |
| EcoR I (--)            | 0.081   | 0.068   |
| Sac I (++)             | 0.300   | 0.364   |
| Msp I (--)             | 0.463   | 0.458   |

Lifestyle Factors

| Diet (mean ± SD) | Men     | Women   |
|------------------|---------|---------|
| carbohydrate (g/day) | 356.9 ± 93.5 | 276.6 ± 88.6 |
| fiber (g/day)     | 4.3 ± 2.0 | 3.9 ± 1.5 |
| n-3 fatty acids (mg/day) | 3.0 ± 1.7 | 2.9 ± 1.3 |
| Keys dietary score | 25.7 ± 8.0 | 29.6 ± 8.6 |

| Alcohol drinking | Men     | Women   |
|------------------|---------|---------|
| >2 drinks/day    | 16 (20.0%) | 2 (2.1%) |
| ≤2 drinks/day    | 24 (30.0%) | 12 (12.6%) |
| non-drinker      | 40 (50.0%) | 81 (85.3%) |

| Cigarette smoking | Men     | Women   |
|-------------------|---------|---------|
| >20 cigarettes/day | 31 (38.8%) | 2 (2.1%) |
| ≤20 cigarettes/day | 14 (17.5%) | 4 (4.2%) |
| non-smoker        | 35 (43.8%) | 89 (93.7%) |

| Physical activity | Men     | Women   |
|-------------------|---------|---------|
| heavy             | 23 (28.8%) | 5 (5.3%) |
| moderate          | 24 (30.0%) | 46 (48.4%) |
| light             | 33 (41.3%) | 44 (46.3%) |

*SD = standard deviation.

Appendix 3. Means and standard deviations of serum lipids and apolipoproteins.

|                      | Men     | Women   |
|----------------------|---------|---------|
| No.                  | 80      | 95      |
| Age (years)          | 55.68 ± 9.11 | 53.31 ± 7.88 |
| TC (mmol/L)          | 5.20 ± 0.99 | 5.26 ± 0.96 |
| LDL-C (mmol/L)       | 3.25 ± 0.94 | 3.31 ± 0.84 |
| TG (mmol/L)*         | 2.47 (0.92−6.67) | 2.15 (0.83−5.55) |
| apo B (mg/dL)        | 101.31 ± 28.45 | 97.61 ± 24.20 |
| HDL-C (mmol/L)       | 1.38 ± 0.33 | 1.47 ± 0.33 |
| apo Al (mg/dL)       | 160.28 ± 28.25 | 165.84 ± 27.14 |
| apo All (mg/dL)      | 37.29 ± 9.21 | 35.57 ± 6.50 |
| apo CIII (mg/dL)*    | 12.88 (6.55−25.35) | 11.75 (5.97−23.12) |

*Medians (95% confidence interval).