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MULTILEVEL ANALYSES OF EFFECTS OF VARIATION IN BODY MASS INDEX ON SERUM LIPID CONCENTRATIONS IN MIDDLE-AGED JAPANESE MEN

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ABSTRACT

For the effective use of the annual workplace health checkup data, we tried to perform multilevel analyses to explore whether the year-to-year weight variation causes any concurrent effects on the lipid profiles among middle-aged Japanese workers. Subjects were 1,939 healthy male workers 40–59 in age from whom serial data of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were collected during health checkups conducted in the years 1997–2000. The effects of body mass index (BMI) on serum concentrations of those lipids were investigated by statistical analysis with multilevel modeling to distinguish multiple levels of information with individual repeated measurements within individuals. A significant increase of TC and TG, and decrease of HDL-C with BMI increase were confirmed. Subanalyses according to both the baseline BMI status (< 25 kg/m^2 or ≥ 25 kg/m^2) and smoking status (never, former, or current) yielded the same BMI-dependent changes of lipid profiles, but obese never smokers failed to show significant effects of BMI on HDL-C concentrations. Multilevel analyses of annual health checkup data linked at individual levels indicated that year-to-year weight variation, though usually in a much narrower range than the between-individual variation, had a strong impact on the corresponding changes of serum concentrations of TC, HDL-C, and TG. This result supports the public health significance of intervention into weight control to prevent the development of atherogenic risks among a healthy workplace population.

Key Words: Multilevel analysis, Random effect, Serum lipid, Weight variation, Industrial health

INTRODUCTION

There have been a number of studies demonstrating the relationship of age and blood lipid profiles.[1-6] A previous cross-sectional study reported age-related changes in lipid profiles in the middle-age range, showing an increase in total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein,

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and triglyceride (TG) levels with age.[1)] In addition, these age-related changes in lipid profiles are also affected by longitudinal weight fluctuations. The Fels Longitudinal Study investigating the association between serial changes in lipid levels and those in body composition among 269 healthy white individuals revealed that changes in adiposity are a more influential independent predictor of longitudinal changes in serum lipid levels than age.[6)] Although the fat and lean tissue can have distinct effects on lipid and lipoprotein levels,[6)] the use of labor-intensive, standardized techniques such as hydrodensitometry is required to evaluate the body composition to attain the requisite level of precision essential to monitoring the longitudinal variation of adiposity over time within individuals. In contrast, the body mass index (BMI), often used as a proxy measure of adiposity and known to be positively related to either TC or TG and negatively related to HDL-C,[7)] is a reproducible anthropometric measurement applicable to mass-screening settings.

Using the weight history of 1,932 male Japanese workers aged 40–59, we have revealed that the root mean square error of variation (RMSE) as a measure of long-term weight fluctuation increases the risk of developing hyperinsulinemia.[8)] While our study focused on the deleterious effects of weight instability during the adulthood period between age 20 and middle-age, the results of some previous studies agree on the beneficial health effects of modest weight loss in terms of the reduced cardiovascular risk or mortality.[9-11)] In this respect, we considered it worthwhile to explore how the yearly weight variation affects the concurrent changes in atherogenic biomarkers, since the health checkup programs are usually conducted on an annual basis in most Japanese workplaces, and the knowledge about the effects of year-to-year weight changes on those biomarkers is thought to constitute a basis for the modification of health behavior. However, the relationship between weight variation and repeatedly measured biomarkers in longitudinal observation has been little investigated, except for the Fels Longitudinal Study which dealt with the follow-up data from a limited number of subjects.[4, 6)]

Generally, the data arising from a longitudinal observation have a complex, correlated structure entailing sources of random variability at multiple levels, which cannot be modeled well by using conventional methods for the analysis of data at a single time point. One of the approaches to handling such hierarchically structured data is multilevel modeling, also know as hierarchical regression, which generalizes ordinary regression modeling to distinguish multiple levels of information in a model.[12)] Multilevel analysis has been used in the fields of education, demography, and sociology to describe an analytical approach that allows the simultaneous examination of the effects of group-level and individual-level variables on individual-level outcomes.[13-16)] Recently, interest in the use of multilevel analysis in medical research has grown.[17-23)] In addition to individuals nested within groups, multilevel models can be applied to other situations with nested sources of random variability, *e.g.* in the analysis of longitudinal data where repeat observations are nested within individuals over time.[24)]

The present study was undertaken to examine the effects of BMI variation on the repeatedly measured serum lipid using a multilevel analysis of data from a large sample of middle-aged Japanese male workers. The data used were regarded as forming a hierarchical structure with individual repeated measurements of serum lipid concentrations (level-1) within individuals (level-2). Because smoking habit is likely to have a certain influence on body weight fluctuations and presents a risk known to elevate serum triglyceride and lower HDL-C,[25)] the interactions between the smoking status and BMI were particularly considered in the analysis.

METHODS

Study population and data collection

The study population was selected from male workers of a manufacturing company in Aichi Prefecture, who agreed to participate in a cohort study of cardiovascular diseases. Women were excluded because of their small number. A total of 2,816 men participated in an annual health checkup program conducted in autumn of the baseline year 1997 and gave written informed consent to answering a lifestyle questionnaire and providing the results of routine biochemical analyses of blood. These analyses were performed in a laboratory which has regularly undergone both the internal and external quality control. We defined eligible subjects as those who were aged 40 to 59 years, completed the lifestyle questionnaire sheet, were not taking medication for diabetes or dyslipidemia, and underwent measurements of all of fasting TC, HDL-C, and TG. These eligibility criteria were met by 1,939 men who were recruited for the current investigation. Serial data of serum lipid and lipoprotein concentrations were collected annually until the health checkup in the year 2000, resignation, retirement, or job transfer, whichever came first, for a total of 7,056 examinations. The study protocol was approved by the Ethical Board of the Nagoya University School of Medicine.

Statistical analysis

Let the random variable Y_{ij} denote the serum lipid measurement at the i_{th} examination for the j_{th} individual. We then assume that Y_{ij} satisfies the following general multilevel model:

Within-individual model - Level 1

$$Y_{ij} = \alpha_j + \beta_j(BMI_{ij} - \overline{BMI_j}) + \gamma_1(age_{ij} - \overline{age_j}) + \gamma_2 Covar_{2j} + \cdots + \gamma_k Covar_{kj} + \varepsilon_{ij}$$

where $i=\{1, 2, 3, 4\}$. Time-varying variables, BMI_{ij} and age_{ij}, are BMI and age at the i_{th} examination for the j_{th} individual, whereas $\overline{BMI_j}$ and $\overline{age_j}$ are the mean values across all examinations for the j_{th} individual. This rescaling of BMI and age so as to center around the individual-specific mean is performed to render the parameters more interpretable.[26] $Covar_{kj}$ is the covariate variable created by dummy-coding of the selected baseline characteristics for the j_{th} individual. Multivariate adjustment was done for age (centered) and the following baseline variables: smoking status (current, former, never), drinking habits (none, light: daily ethanol consumption approximately less than 23 g; moderate: 23 to 46 g; heavy: 46 g or over), leisure-time physical activity (not very active, somewhat active, regularly active), preference for fatty taste (yes, no), and presence of a family history of dysplipidemia among siblings or parents for the j_{th} individual. The classification method for the intensity of both drinking habits and leisure-time physical activity has been thoroughly described in our previous paper.[27] The intercept α_j represents the average measurement of the lipid profiles for the j_{th} individual with average age and BMI across all of the subject's examinations, and ε_{ij} the error components which account for the within-individual variability. The regression coefficient β_j is used to model the linear variation of serum lipid concentrations with BMI.

Random effects were added to reflect the natural heterogeneity in the population. In this model, both the intercept and the slope for time were allowed to vary across individuals, and the individual-specific regression coefficients were defined at the second level:

Subject random-intercept-slope model - Level 2

$$\alpha_j = \alpha + \upsilon_j$$
$$\beta_j = \beta + \varpi_j$$

The random components, υ_j and ϖ_j, measure the variation of individual's mean measurement of

the lipid concentrations and slope, respectively, from their average in the whole sample.
For the statistical software, we ran the Statistical Analysis Package (SAS) release 9.1 licensed to the Nagoya University Information Technology Center. Multilevel model fitting was performed using the procedure PROC MIXED, where we specified RANDOM statement and type=UNSTRUCTURED, which does not assume the random-effects covariance to be of any specific form.[24,28,29] These multilevel analyses were conducted for all subjects in the beginning, and subanalyses were done for subjects with baseline BMI < 25 kg/m^2 (non-obese group) and those with BMI ≥ 25 kg/m^2 (obese group). Further subanalyses were done by smoking status.

To consider the interaction between the smoking habit and weight, additional subanalyses were conducted by smoking status in obese vs. non-obese group, separately. Since fasting TG was found to be skewed in the distribution of our study population,[30] a logarithmic transformation was adopted for normalization in all multilevel analyses. All *p* values for statistical tests were two-tailed, and values < 0.05 were regarded as statistically significant.

RESULTS

The number of subjects who participated in the annual health checkup conducted in the year 1998, 1999, and 2000 declined to 1,860, 1,714, and 1,543, respectively, mostly due to compulsory retirement. Table 1 gives the baseline characteristics of the 1,939 subjects. Age and BMI averaged 51.4 years and 22.7 kg/m^2, respectively, and those with BMI ≥ 25 kg/m^2 accounted for 19.4% in this study population. With respect to dyslipidemic profiles at baseline, 15.2%, 16.0%, and 26.1% were subjects with TC ≥ 240 mg/dl, HDL-C < 40 mg/dl, and TG ≥ 150 mg/dl, respectively.

Estimated fixed-effects of age and BMI on TC, HDL, and TG concentrations are presented in Table 2. As BMI increases, significant increases in TC and TG, and significant decreases in HDL-C were observed. With age, both TC and HDL-C significantly increased, whereas TG significantly decreased. The estimated random-effects shown in Table 2 indicated that variability in the individual's mean of TC, HDL-C, and TG between subjects significantly contributes to the population variability. The slope, which refers to the rates of change in HDL-C and TG concentrations to BMI, also significantly varied between subjects, but TC did not have a significantly variable slope between subjects.

In the subanalyses according to the baseline BMI status, the concentrations of TC and TG were positively associated with BMI both in the non-obese and obese group, while those of HDL-C were negatively associated with it, as shown in Table 3. The results showed that variability in the intercept in all of the models and variability in the slope in most of the models also significantly contributed to the population variability. Similar significant relationships of BMI with the serum lipid concentrations, and significant between-subject variability in the intercept were demonstrated irrespective of the smoking status (Table 4).

Table 5 showed that, in never-smokers, the fixed effects of BMI on the HDL-C concentrations were significant in the non-obese subpopulation, but not significant in the obese subpopulations. On the other hand, in current smokers, BMI was significantly related to the HDL-C concentrations, irrespective of the baseline BMI status. However, the relationship between BMI and TG concentrations was significant in both the non-obese and obese never smokers (data not shown).

Table 1 Basic characteristics of 1,939 men at baseline

	Average	Median	(Min, Max)
Age (year)	51.4	52	(40, 59)
Body mass index (kg/m^2)	22.7	22.6	(14.8, 43.3)
Total cholesterol (mg/dl)	206.4	204	(82, 355)
HDL cholesterol (mg/dl)	53.3	51	(25, 126)
Triglyceride (mg/dl)	128.1	108	(29, 1049)
	No. of subjects		(%)
Age			
≥ 50 years		1,308	(67.5)
Body mass index			
≥ 25 kg/m^2		377	(19.4)
Smoking status			
Never		694	(35.8)
Past		56	(2.9)
Current		1,189	(61.3)
Drinking status#			
None		494	(25.5)
Light		394	(20.3)
Moderate		531	(27.4)
Heavy		520	(26.8)
Physical activity			
Not very active		1,162	(59.9)
Somewhat active		691	(35.6)
Regularly active		86	(4.5)
Family history of dyslipidemia			
Yes		67	(3.5)

Light: daily ethanol consumption of approximately < 23 g; moderate: ≥ 23 g, < 46 g; heavy: ≥ 46 g.

Table 2 Parameter estimates from mixed model describing effect of BMI variation on longitudinal lipid profiles of 1,939 men

Dependent variable	Total cholesterol		HDL cholesterol		Triglyceride#	
	Parameter estimates*	*p* value	Parameter estimates*	*p* value	Parameter estimates*	*p* value
Fixed effects estimates						
BMI+ (kg/m^2)	3.6	< 0.01	-1.3	< 0.01	0.098	< 0.01
Age+ (year)	2.1	< 0.01	1.4	< 0.01	-0.0084	0.032
Random effects estimates						
Variance of intercept	888.5	< 0.01	161.0	< 0.01	0.21	< 0.01
Variance of slope	7.1	0.094	4.0	< 0.01	0.0058	< 0.01
Covariance	7.0	0.52	-3.3	0.06	0.0042	0.20

Log-transformed.
* Adjusted for age, preference for fatty taste, smoking status, drinking status, leisure time physical activity, and family history of dyslipidemia.
+ Variables rescaled to center around the subject-specific mean.

Table 3 Parameter estimates from mixed model describing effect of BMI variation on longitudinal lipid profiles of 1,939 men by baseline BMI level

Dependent variable	Total cholesterol		HDL cholesterol		Triglyceride#	
	Parameter estimates*	*p* value	Parameter estimates*	*p* value	Parameter estimates*	*p* value
			BMI < 25 (*n* = 1,562)			
Fixed effects estimates						
BMI+ (kg/m^2)	3.5	< 0.01	-1.3	< 0.01	0.090	< 0.01
Age+ (year)	2.4	< 0.01	1.4	< 0.01	-0.0058	0.19
Random effects estimates						
Variance of intercept	875.4	< 0.01	163.6	< 0.01	0.20	< 0.01
Variance of slope	13.1	0.024	5.3	< 0.01	0.0064	< 0.01
Covariance	15.9	0.20	-2.3	0.28	0.0037	0.31
			BMI ≥ 25 (*n* = 377)			
Fixed effects estimates						
BMI+ (kg/m^2)	3.5	< 0.01	-1.2	< 0.01	0.12	< 0.01
Age+ (year)	0.80	0.11	1.5	< 0.01	-0.017	0.045
Random effects estimates						
Variance of intercept	893.3	< 0.01	117.8	< 0.01	0.18	< 0.01
Variance of slope	0.0	-	0.89	0.014	0.0046	0.077
Covariance	-	-	-6.2	0.16	0.00068	0.92

Log-transformed.

* Adjusted for age, preference for fatty taste, smoking status, drinking status, leisure time physical activity, and family history of dyslipidemia.

+ Variables rescaled to center around the subject-specific mean.

Table 4 Parameter estimates from mixed model describing effect of yearly BMI variation on longitudinal lipid profiles of 1,939 men by smoking status

Dependent variable	Total cholesterol		HDL cholesterol		Triglyceride#	
	Parameter estimates*	*p* value	Parameter estimates*	*p* value	Parameter estimates*	*p* value
			Never smoker (*n* = 694)			
Fixed effects estimates						
BMI+ (kg/m^2)	3.1	< 0.01	-1.4	< 0.01	0.096	< 0.01
Age+ (year)	2.2	< 0.01	1.6	< 0.01	-0.0079	0.22
Random effects estimates						
Variance of intercept	890.9	< 0.01	166.4	< 0.01	0.20	< 0.01
Variance of slope	0.0	-	6.7	< 0.01	0.012	< 0.01
Covariance	-	-	-1.3	0.69	0.013	0.035
			Former smoker (*n* = 56)			
Fixed effects estimates						
BMI+ (kg/m^2)	2.6	0.21	-2.2	< 0.01	0.076	< 0.01
Age+ (year)	2.6	0.032	1.1	< 0.01	-0.018	0.32
Random effects estimates						
Variance of intercept	680.6	< 0.01	172.4	< 0.01	0.20	< 0.01
Variance of slope	24.0	0.24	5.1	0.18	0.0	-
Covariance	139.0	0.050	-3.3	0.75	-	-
			Current smoker (*n* = 1,189)			
Fixed effects estimates						
BMI+ (kg/m^2)	3.8	< 0.01	-1.2	< 0.01	0.10	< 0.01
Age+ (year)	2.0	< 0.01	1.4	< 0.01	-0.0084	0.097
Random effects estimates						
Variance of intercept	897.8	< 0.01	156.8	< 0.01	0.21	< 0.01
Variance of slope	12.8	0.032	2.3	< 0.01	0.0037	0.058
Covariance	-12.8	0.38	-4.3	0.036	0.0011	0.79

Log-transformed.

* Adjusted for age, preference for fatty taste, drinking status, leisure time physical activity, and family history of dyslipidemia.

+ Variables rescaled to center around the subject-specific mean.

Table 5 Fixed-effects estimates from mixed model describing effect of BMI variation on HDL-cholesterol concentrations of 1,939 men by smoking status at different baseline BMI levels

	Parameter estimates*	*p* value
BMI < 25 kg/m^2		
Never smoker (*n* = 549)	-1.6	< 0.01
Former smoker (*n* = 45)	-1.9	0.059
Current smoker (*n* = 968)	-1.1	< 0.01
BMI ≥ 25 kg/m^2		
Never smoker (*n* = 145)	-0.76	0.13
Former smoker (*n* = 11)	-4.4	0.035
Current smoker (*n* = 221)	-1.1	< 0.01

* Adjusted for age (rescaled to center), preference for fatty taste, drinking status, leisure time physical activity, and family history of dyslipidemia.

DISCUSSION

In this study of middle-aged men, longitudinally observed lipid data with concurrent measure of obesity were analyzed using multilevel analysis. As is usual in repeated measurements of serum biomarkers, the between-individual variation is much wider than the within-individual one, which is likely to compromise the statistical power when conventional regression analyses are applied to data from repeated measurements nested within individuals over time. A strong point of the multilevel model is that it enables one to adjust for the influence of a wide variety of correlation structures and to estimate such parameters of particular interest as the variance and covariance.[24] In this respect, the results of our study indicated a wide variation of individual-specific mean serum concentrations of TC, HDL-C and TG. The individual-specific slopes describing the rates of change in serum lipid concentrations to BMI were also found to vary widely from subject to subject.

Overweight and obesity are considered underlying causes of elevated serum TG and low HDL-C in the general population.[25] Moreover, a positive relationship of weight with TC or TG, and a negative relationship with HDL-C were confirmed even in a lean Asian population.[7] The effects of weight loss in obese individuals have also been systematically reviewed.[31] Although long-term beneficial effects of weight reduction on TC or TG levels were consistently demonstrated in previous reports, the systematic review revealed an extreme variation in the HDL-C response to weight reduction, concluding that HDL-C has a poor relationship with weight loss compared to the other lipid levels.

One of the salient results of this study is that significant relationships of BMI with the serum concentrations of TC, HDL-C and TG were confirmed using data from longitudinal observations. Because the multilevel modeling allows one to distinguish the effect of wide between-subject variability in both the intercepts and slopes, the observed relationship of BMI to the lipid concentrations suggested that the year-to-year BMI variations have a significant impact on the lipid profiles. The annual workplace health checkup is mandated by the Industrial Safety and Health Law of Japan, and the effective use of annually collected data in linkage at the individual level has been our great interest. The results of our study indicated that prompt improvements in

lipid profiles are expected to occur with mild weight control even from yearly observation, while poor weight control is likely to result in immediate worsening of lipid profiles at the same time. Similar results are universally confirmed in the subanalyses, irrespective of either the baseline BMI status or smoking status. We consider that the characterization of the relationship between BMI and lipid profiles indicated in the present study has significant ramifications for public health from the practical point of view, since the results can be incorporated into workplace health promotion programs to raise awareness of the importance of weight control. The biological explanation for the lack of significant BMI-dependent changes in HDL-C concentrations among obese never smokers remains to be elucidated in further investigations. In light of a previous report which revealed that HDL-C is not as sensitive as other serum lipid levels in terms of response to weight variation,[30] the involvement of factors not identified in our study may well play some role in this particular subpopulation. Meanwhile, LDL-C was not included as part of the lipid profiles in this study, since its serum concentration was not directly determined. We thus estimated LDL-C concentrations using Friedewald's formula and found that LDL-C behaved in a similar way as TC in both the main analysis and subanalysis models (data not shown).

Our study has a number of limitations. Our reason for treating age and BMI as time-varying in the model was to better evaluate patterns of changes in lipid profiles with these predictors. However, we did not treat other covariates as time-varying, because information on those covariates was not available on a year-to-year basis. The possibility can not be ruled out therefore that the classification of such lifestyle variables as smoking/drinking habit or physical activity could have changed in an individual during the serial data collection. Moreover, while those under medical treatment for diabetes or dyslipidemia at baseline were excluded from the analyses, our study may be affected by the cases who started to undergo medical treatment anew during the follow-up period. In our rough estimation based on the intermediate evaluation, 7% of our study population were newly diagnosed with dyslipidemia during the years 1997–2004, indicating an annual incidence rate of about 1–2%. The influence of those cases also remains to be evaluated in any further analyses.

Another limitation is the unbalanced nature of our data in the sense that an equal number of measurements were not available for all subjects, mostly due to compulsory retirement in the course of follow-up, and less often, to resignation, job transfer, or simply skipping the annual health checkup. Multilevel analysis uses all the available information of the incomplete data without the need either to delete or to impute measurements.[29] While this is justified whenever the missing data mechanism is assumed to occur at random, no such assumption seemed warranted with respect to the dropout process in our data. In fact, we have observed approximately 150 yearly retirements from our study population, which accounted for most of the missing, and such nonrandom dropouts are likely to occur among the older subjects.

In conclusion, it is suggested that multilevel analyses are a useful analytic tool for effective use of the serial annual health checkup data linked at individual levels. Even though the year-to-year weight variation within individuals is usually confined to a much narrower range than the between-individual variation, its impact on the corresponding yearly changes of serum concentrations of TC, HDL-C, and TG is found to be strong, underscoring the importance of workplace health promotion programs aimed at proper weight control to achieve improvements in lipid profiles.

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