Original Article

Seasonal Variation in Serum Lipid Levels in Japanese Workers

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Aim: Seasonal variation in serum lipid levels in the Japanese population remains unclear. The aim of this study was to determine whether a variation in lipid levels exists in Japanese workers.

Methods: We investigated 1,331 employees in our institution (1,192 men, 44 ± 10 years; 139 women, 38 ± 11 years) who underwent health checkups in both June (summer) and December (winter), 2008.

Results: Serum levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglyceride were significantly higher in winter than in summer (129.1 ± 31.2 mg/dL versus 125.2 ± 30.2 mg/dL, $p < 0.0001$; 65.9 ± 16.8 mg/dL versus 63.5 ± 16.1 mg/dL, $p < 0.0001$; 110.4 ± 67.5 mg/dL versus 107.5 ± 70.4 mg/dL, $p < 0.05$; respectively), although the ratio of LDL to HDL cholesterol was comparable (2.11 ± 0.81 in summer versus 2.12 ± 0.81 in winter). The frequency of study subjects diagnosed with hypercholesterolemia, defined as LDL cholesterol ≥ 140 mg/dL, was significantly higher in winter than in summer (34.5 % versus 30.9 %, $p < 0.0001$).

Conclusion: In Japanese workers, we demonstrated that there is a seasonal variation in serum lipid levels and the prevalence of hypercholesterolemia. This result indicates that we have to give careful consideration to the season of blood sampling in the clinical diagnosis of and management decisions for hypercholesterolemia.

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Key words; Seasonal variation, Lipid, Low-density lipoprotein cholesterol, Direct method, Japanese workers

Introduction

It is well recognized that elevated levels of low-density lipoprotein (LDL) cholesterol play a central role in both the pathogenesis and development of atherosclerosis, as well as in the clinical consequences, such as myocardial infarction, stroke, peripheral vascular disease, and heart failure¹. It is clear that elevated levels of LDL cholesterol are a major predictor of cardiovascular disease (CVD). In addition, a number of large randomized controlled trials have demonstrated that LDL cholesterol lowering with 3-hydroxy-3-

methylglutaryl coenzyme A reductase inhibitor (statin) therapy can substantially reduce CVD-related morbidity and mortality²⁻⁵. Thus, recent treatment guidelines, such as the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), have been proposed for LDL cholesterol levels based on the number of risk factors⁶; however, the guidelines do not take into consideration that seasonal variation in serum LDL cholesterol levels may affect clinical management.

Accumulating evidence has demonstrated that there is a seasonal variation in serum lipid levels, although its mechanism is not fully understood⁷⁻⁹. The variation usually has a tendency to be higher in fall and winter than in spring and summer. A previous study in Finland suggests that there may be as much as a 100-mg/dL seasonal variation in serum cholesterol levels¹⁰. A cross-sectional study has reported that the frequency of male subjects having hypercholester-

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olemia, defined as total cholesterol ≥ 240 mg/dL, was 25.4 % in winter and 13.5 % in summer¹¹). These findings indicate that seasonal variation may be taken into account in clinical lipid management.

To our knowledge, the seasonal variation in lipid levels, especially serum LDL cholesterol levels by a direct method, in the Japanese population remains unclear. Therefore, the aim of this study was to determine whether a variation in serum lipid levels exists in Japanese workers.

Methods

This study was conducted in accordance with the principles expressed in the Declaration of Helsinki. The study protocol was approved by the Committee of the Nuclear Science Research Institute, Tokai Research and Development Center, Japan Atomic Energy Agency.

Study Subjects

We investigated 1,331 employees (1,192 men, 44 ± 10 years; 139 women, 38 ± 11 years) belonging to our institution, who underwent health checkups in both June (summer) and December (winter), 2008. In this study, we did not enroll workers who were taking lipid-lowering medicines including statins, because the study results are highly influenced by patient compliance. No participants had a history of hepatobiliary disease, coronary artery disease, stroke, or cancer.

Health Checkups

Demographic data and health information were collected by a self-administered questionnaire, and all subjects underwent a serological test and physical examination, including blood pressure, height, and weight at each checkup. Venous blood samples were collected from each subject after 9 hours or overnight fasting. Using automatic measurements devices, serum LDL cholesterol (enzymatic method), high-density lipoprotein (HDL) cholesterol (direct method), and triglyceride (TG) (enzymatic method) concentrations were measured.

Direct Measurements of Serum LDL Cholesterol Levels

LDL cholesterol levels have been bound for direct measurement instead of total cholesterol levels in health checkups at our institution since April, 2008. In the checkups, serum LDL cholesterol levels are measured directly by a liquid reagent system (Determiner L; Kyowa Medex). This assay, based on a cholesterol assay system comprising cholesterol esterase

Table 1. Characteristics of Study Subjects

Characteristic	<i>n</i> = 1,331
Age, y	43 $\pm$ 10
Gender (male/female, <i>n</i>)	1,192/139
Body mass index, kg/m ²	23.6 $\pm$ 3.0
Obesity, <i>n</i> (%)	347 (26.1)
Systolic blood pressure, mm Hg	124 $\pm$ 14
Diastolic blood pressure, mm Hg	70 $\pm$ 11
Fasting glucose, mg/dL	88 $\pm$ 13
Current smoker, <i>n</i> (%)	287 (21.6)
Medication, <i>n</i> (%)	
Hypertension	104 (7.8)
Diabetes mellitus	21 (1.6)

Data are expressed as the mean $\pm$ SD or number (%). *n* indicates subject number. Obesity is defined as body mass index ≥ 25 kg/m².

and cholesterol oxidase, uses a surfactant capable of selectively solubilizing LDL, and guides esterified cholesterol and free cholesterol contained in LDL to the cholesterol reaction system to determine the content of LDL cholesterol. Inter-assay coefficients of variation are less than 5 %. This assay shows high reactivity to intermediate-density lipoprotein (IDL) cholesterol¹²).

Statistical Analysis

Continuous data are expressed as the mean $\pm$ SD. Categorical data are presented as absolute values and percentages. Continuous variables were compared by a paired *t* test. Chi-square tests were used to compare categorical variables. The value of *p* < 0.05 was considered to be significant. Statistical analysis was performed using the JMP Statistical Discovery Software for Windows version 7 (SAS Institute Inc., Cary, USA).

Results

Characteristics of Study Subjects

The characteristics of the study population in summer are shown in **Table 1**. The frequency of obese subjects, defined as body mass index ≥ 25 kg/m², was 26.1 % (*n* = 347). Mean values of systolic and diastolic blood pressure, and fasting glucose were within the normal range. Current smokers were 21.6 % in the study population, and study subjects receiving medicines for hypertension were 7.8 % (*n* = 104) and for diabetes mellitus were 1.6 % (*n* = 21). Body mass index was comparable in summer and winter (data not shown). Systolic and diastolic blood pressure ($124 \pm 14/70 \pm 11$ mm Hg in summer versus $128 \pm 15/77 \pm$

Table 2. Seasonal Variation in Lipid Levels

	Summer	Winter	<i>p</i> value
LDL cholesterol, mg/dL	125.2 ± 30.2	129.1 ± 31.2	< 0.0001
HDL cholesterol, mg/dL	63.5 ± 16.1	65.9 ± 16.8	< 0.0001
LDL-HDL ratio	2.11 ± 0.81	2.12 ± 0.81	0.8681
Triglyceride, mg/dL	107.5 ± 70.4	110.4 ± 67.5	0.0237

All data are expressed as the mean ± SD. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Fig. 1. Frequency of study subjects having hypercholesterolemia, defined as LDL cholesterol ≥ 140 mg/dL, was significantly higher in winter (34.5 %) than in summer (30.9 %)

Data are presented as percentages. * $p < 0.0001$ by chi-square test.

11 mm Hg in winter), and fasting glucose (88 ± 13 mg/dL in summer versus 90 ± 16 mg/dL in winter) were markedly different in both seasons.

Seasonal Variation in Lipid Levels

Complete lipid measurement data were obtained from all subjects. Serum levels of LDL and HDL cholesterol, and TG, and the ratio of LDL to HDL cholesterol (LDL-HDL ratio) in summer and winter are shown in **Table 2**. Serum levels of LDL and HDL cholesterol, and TG were significantly higher in winter than in summer, although the LDL-HDL ratio was comparable in both seasons ($p = 0.8681$). The frequency of study subjects diagnosed with hypercholesterolemia, defined as LDL cholesterol ≥ 140 mg/dL, was significantly higher in winter than in summer (34.5 % versus 30.9 %, $p < 0.0001$) (**Fig. 1**). The frequency of study subjects diagnosed with dyslipidemia, defined as HDL cholesterol < 40 mg/dL or TG ≥ 150 mg/dL, was similar in both seasons (data not shown).

Influence of Age, Gender and Obesity on LDL Cholesterol Levels

Serum LDL cholesterol levels in summer and winter are shown according to age, in **Table 3**. LDL cholesterol levels in both seasons increased with age up to 40 years and the data were significantly higher in winter than in summer in all age groups. In male subjects, serum LDL cholesterol levels increased from 126.7 ± 29.7 mg/dL in summer to 130.5 ± 30.9 mg/dL in winter ($p < 0.0001$). In female subjects, serum LDL cholesterol levels elevated from 112.8 ± 31.3 mg/dL in summer to 117.0 ± 31.0 mg/dL in winter ($p < 0.0001$). This variation occurred irrespective of obesity. In obese subjects, serum LDL cholesterol levels increased from 135.6 ± 28.9 mg/dL in summer to 139.6 ± 30.3 mg/dL in winter ($p < 0.0001$). In non-obese subjects, serum LDL cholesterol levels elevated from 121.6 ± 29.8 mg/dL in summer to 125.4 ± 30.6 mg/dL in winter ($p < 0.0001$). Moreover, there was a similar variation independent of the smoking status (data not shown).

Seasonal Variation in Hematologic Parameters

We examined hematologic parameters, because a previous report suggests that changes in blood volume acclimated to season may explain the seasonal variation in lipid levels¹³). Levels of hemoglobin and hematocrit were significantly higher in winter than in summer (14.9 ± 1.2 g/dL versus 14.7 ± 1.2 g/dL, $p < 0.0001$; 44.3 ± 3.5 % versus 44.0 ± 3.3 %, $p < 0.0001$; respectively). The ratio of LDL cholesterol to hemoglobin and to hematocrit was significantly higher in winter than in summer (8.73 ± 2.15 versus 8.54 ± 2.12 , $p < 0.0001$; 2.92 ± 0.71 versus 2.85 ± 0.69 , $p < 0.0001$; respectively) (**Table 4**).

Discussion

In the present study, we demonstrated that there is a seasonal variation in LDL and HDL cholesterol, and TG levels and the prevalence of hypercholesterolemia in Japanese workers. This result indicates that the season of serum lipid measurements may affect the

Table 3. Influence of Age on LDL Cholesterol Levels

Age, y	Summer	Winter	<i>p</i> value
≥ 50 (<i>n</i> = 404)	130.9 ± 29.2	133.6 ± 30.4	0.0005
40–49 (<i>n</i> = 427)	129.6 ± 29.2	134.5 ± 29.6	< 0.0001
30–39 (<i>n</i> = 374)	121.0 ± 30.0	125.1 ± 31.7	< 0.0001
< 30 (<i>n</i> = 126)	104.9 ± 26.0	108.3 ± 27.0	0.0081

Data are expressed as the mean ± SD. *n* indicates subject number.

Table 4. Seasonal Variation in Hematologic Parameters

	Summer	Winter	<i>p</i> value
Hemoglobin, mg/dL	14.7 ± 1.2	14.9 ± 1.2	< 0.0001
Hematocrit, %	44.0 ± 3.3	44.3 ± 3.5	< 0.0001
LDL-Hemoglobin ratio	8.54 ± 2.12	8.73 ± 2.15	< 0.0001
LDL-Hematocrit ratio	2.85 ± 0.69	2.92 ± 0.71	< 0.0001

All data are expressed as the mean ± SD. LDL, low-density lipoprotein.

clinical diagnosis and management decisions. It also shows that the variation in serum LDL cholesterol levels may exist independently of age, gender, obesity, and smoking status.

It is well accepted that lipid management, especially LDL cholesterol management, is very important for the pathogenesis and/or development of CVD. Thus, the NCEP-ATP III and other guidelines recently proposed treatment goals of LDL cholesterol levels based on patients' risk for cardiovascular and metabolic problems that determine the intensity of lipid-lowering therapy^{6, 14, 15}. In Japan, LDL cholesterol levels are bound for measurement in health checkups, which started in 2008 to prevent lifestyle-related diseases, with the focus on the metabolic syndrome. In this study, LDL cholesterol levels were directly measured by a liquid reagent system (Determiner L; Kyowa Medex), although direct methods have several problems such as fluctuation of data among methods. This data discrepancy is derived from the reactivity to IDL cholesterol, which is a strong risk factor for atherosclerosis, among methods. The assay in this study includes LDL cholesterol and the assay data have been proved to correlate well with those of the Friedewald calculation and ultracentrifugal assay. So far, the Friedewald calculation has been used in almost all studies of seasonal variation in serum LDL cholesterol levels, but the calculation has the fundamental flaw that the method yields a relatively reliable estimate only when TG levels are lower than 200 mg/dL¹⁶. In addition, LDL cholesterol levels cannot always be accurately evaluated when TG levels exceed 400 mg/dL. It is also necessary to obtain serum after at least 12 hours of

fasting in order to precisely assess TG levels. Moreover, although the ultracentrifugal assay is the accepted a reference method, it requires relatively large volume of serum, long turnaround time, and is unsuitable for routine usage, such as health checkups; therefore, direct measurements of LDL cholesterol levels have recently been applied for routine usage and to replace the calculation. To our knowledge, seasonal variations in lipid levels, especially LDL cholesterol levels by a direct method, in the Japanese population remain unclear, so we examined whether the variation exists in Japanese workers.

This study demonstrated the seasonal variation in serum lipid levels, and 3.6 % more subjects had hypercholesterolemia, defined as LDL cholesterol ≥ 140 mg/dL, in winter. We suppose that this finding may provide important information in clinical practice. First, the seasonal variation in serum LDL cholesterol levels may be considered in clinical management in the Japanese population, although it is low. It is reported that the seasonal variation in serum LDL cholesterol levels may play an important role in the seasonal incidence of CVD events^{17, 18}. In this study, LDL cholesterol levels were significantly higher in winter than in summer, but the LDL-HDL ratio, which is a strong predictor of atherosclerosis^{19, 20}, was comparable in both seasons. Further studies are needed to clarify its clinical significance with CVD events. The second is that the season of blood sampling may produce misclassification for hypercholesterolemia, which is connected with delay of therapeutic intervention. This study suggests that season of lipid measurements in subjects who are obese, current smoker, or

aged ≥ 40 years should be taken into account in clinical practice. This result supports the importance of lifestyle management (e.g., weight reduction, diet modification, and smoking cessation) in clinical practice.

The mechanism for seasonal variation in serum lipid levels is not fully understood. Studies examining the role of the body mass index, diet, and physical activity have reported that these variables did not fully explain the variation between seasons^{11, 21)}. On the other hand, Robinson demonstrated that mean monthly cholesterol levels were negatively correlated with mean monthly air temperatures, although the seasonal differences were observed independent of changes in body weight²²⁾. Ockene reported a possible mechanism that the change in blood volume acclimated to environmental temperature and/or physical activity can be contributed to an accompanying variation in lipid levels¹³⁾. Based on the meteorological data obtained from the Mito Local Meteorological Observatory, mean monthly air temperature in this area was 19.0°C in June and 1.7°C in December, 2008. This study demonstrated that hemoglobin and hematocrit levels, and the ratio of LDL cholesterol to hemoglobin and to hematocrit were significantly higher in winter than in summer. This result suggests that one of the mechanisms may be independent of the change in blood volume. Differences in the diet and physical activities of study participants between summer and winter may have some influence. We hope that these mechanisms will be clarified in the future.

There are some limitations to this study. First, our study was limited to a single year and extended observation will be necessary in the near future. Second, there was the small number of female subjects, which was too small to examine the seasonal variation in lipid levels in Japanese female workers, although we provided some evidence of a significant outcome. Third, we could not investigate differences in the diet and physical activities of the study subjects, which are very important for better understanding the mechanisms of the seasonal variation in lipid levels. Last, it could not be demonstrated whether the variation has a role in the clinical setting. Further studies are therefore needed to clarify its clinical importance.

In conclusion, we demonstrated in Japanese workers that there is seasonal variation in serum lipid levels and the prevalence of hypercholesterolemia. This result indicates that the season of lipid measurements may affect risk assessments, clinical diagnosis and management decisions in clinical practice.

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