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Original Article

Serum Total Cholesterol of New Students Enrolled at Okayama University: Trend during 1989-1998

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To clarify the trend of hypercholesterolemia in Japanese adolescents, we investigated the serial changes in body mass index (BMI) and serum total cholesterol (TC) concentrations among 5,700 new students enrolled at Okayama University in 1989, 1993, and 1998. After confirming the stability of the TC assay of serum samples stored at −80 °C, we measured serum TC levels in stored serum samples using an automated assay system. Although serum TC levels were higher in females than in males, these levels correlated weakly and positively with BMI ($r = 0.21$, $P < 0.001$) in males but not in females. Serum TC concentrations progressively increased from 1989-1998 in both sexes, irrespective of changes in BMI. In subjects with normal BMI (≥ 19 and < 23 kg/m^2), a significant increase in serum TC was noted from 1989-1998 in both males (157.2 ± 1.0 to 163.6 ± 0.9 mg/dl) and females (172.0 ± 1.1 to 175.6 ± 1.0 mg/dl). Our results indicate on increased incidence of hypercholesterolemia even in nonobese young Japanese adolescents. A concerted effort by health and education officials together with parents is necessary to prevent a further rise in the incidence of hypercholesterolemia among young Japanese.

Key words: lifestyle-related disease, masked obesity, adolescent, body mass index

There is a general belief that the incidence of lifestyle-related diseases (LRD) has increased in recent decades, especially in developed countries. Of the various LRD, hyperlipidemia is considered to be one of the most critical risk factors for coronary heart diseases [1, 2] and to relate to obesity. In Japan, the mean concentration of serum total cholesterol (TC) increased by 8 and 10 mg/dl from 1960 to 1980 in males and females, respectively [3-5], though it remained unchanged from 1980 to 1990 [6]. Nevertheless, because the proportion of individuals with hyperlipidemia is suspected to have increased in Japan in recent years, such a rise is a major public health problem that may contribute to increased morbidity and mortality. A suitable and concerted effort should therefore be made to prevent further rises in hypercholesterolemia.

We have recently reported that the population of obese male Japanese adolescents has increased during the last 40 years, despite body weight in females having been maintained [7], and that the increment in body weight closely correlates with future morbidity [8]. The link between child and adult obesity has previously been investigated by several groups [9-11], with their reports suggesting that even at a young age, obesity is associated with increased morbidity and mortality. Although synchronic studies have shown the trend of rising serum cholesterol levels among adults [3-6], long-term changes in serum cholesterol levels have not been carefully inves-

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tigated in a similar group of young adolescents.

At our Health and Medical Center, serum TC has been measured at matriculation health examination since 1989 (Table 1). Nevertheless, we cannot determine the actual trend of hypercholesterolemia among young university students form these data, since the assay method for measuring TC has been modified 3 times during the last 10 years and the average of body mass index (BMI) was diverse in each entrance year.

In the present study, we investigated the serial changes in BMI and serum TC over 10 years among newly enrolled students at Okayama University in 1989, 1993, and 1998. For this purpose, we used the health-related data recorded at the entrance health examination that was available from the Health and Medical Center, and determined serum TC by the same assay using the preserved sera. The intent of our study was to determine the actual trend in serum TC levels in recent years in young Japanese University students. Such data should be useful in designing strategies that prevent LRD in university students.

Subjects and Methods

Subjects. We first examined the effect of storage of blood samples on measurement of serum TC. We collected the remaining 1 ml serum, which was obtained at matriculation from each student upon their health examination at our university, and preserved the samples at $-80\,^{\circ}C$ for a maximum of 10 years. We selected each of the 30 samples after being preserved for 1, 3, 5, 6, 8, and 10 years, respectively, without liquefaction. These samples were selected from subjects whose serum TC levels were recorded at the Health and Medical Center. They were also selected randomly from each of the 10 samples of low (< 150 mg/dl), middle (≤ 150 and < 220 mg/dl), and high (≥ 220 mg/dl) serum TC concentrations.

The subjects of our study were 5,700 newly enrolled students (male, 3,656; female, 2,044) at Okayama University, including 1,713 from 1989 (male, 1,102; female, 611), 1,781 from 1993 (male, 1,155; female, 626), and 2,206 from 1998 (male, 1,399; female, 807). The age of these students ranged from 18 to 19 years in each year-group. Our sample was selected from a total of 6,797 newly enrolled students, representing an inclusion rate of 84.4 and 82.9% for males and females, respectively. The inclusion criteria were the availability of health-related data and stable storage of sera in the freezer in our institution.

Methods. In a preliminary study, the original values of serum TC measured using assay kits different from the present one were converted according to the formula ($y = 0.976x + 7.71$ for 1989; $y = 1.00x + 4.09$ for 1993), and compared with the present measurements assayed by the unified method (see below). Based on the results of the preliminary study, we compared the serial changes in serum TC concentration and the relationship between BMI and serum TC recorded for each year for males and females. In this study, a BMI value of 21 kg/m^2 was regarded as the standard BMI, since the average of BMI of newlyenrolled male and female stu-

Table 1 Serial changes in BMI and total cholesterol (TC) concentrations in all subjects undergoing the matriculation health examination from 1989 to 1998

Entrance	Males				Females			
	BMI (kg/m^2)		Serum TC (mg/dl)		BMI (kg/m^2)		Serum TC (mg/dl)	
year	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n
1989	21.2 ± 0.1	1,373	158.0 ± 0.8	1,407	20.8 ± 0.1	668	172.0 ± 1.4	707
1990	21.7 ± 0.1	1,516	163.2 ± 0.7	1,515	20.7 ± 0.1	684	175.9 ± 1.2	683
1991	21.5 ± 0.1	1,457	166.5 ± 0.8	1,177	20.9 ± 0.1	726	176.1 ± 1.2	607
1992	21.7 ± 0.1	1,486	167.2 ± 0.8	1,475	20.7 ± 0.1	757	183.3 ± 1.2	739
1993	21.5 ± 0.1	1,416	165.7 ± 0.7	1,414	20.7 ± 0.1	902	177.3 ± 1.0	899
1994	21.6 ± 0.1	1,480	164.5 ± 0.7	1,506	20.7 ± 0.1	809	174.7 ± 1.0	809
1995	21.6 ± 0.1	1,417	169.4 ± 0.8	1,422	20.8 ± 0.1	857	180.8 ± 1.0	858
1996	21.3 ± 0.1	1,434	169.1 ± 0.7	1,430	20.3 ± 0.1	892	180.9 ± 1.0	895
1997	21.1 ± 0.1	1,428	164.8 ± 0.8	1,425	20.3 ± 0.1	861	177.6 ± 1.0	858
1998	21.3 ± 0.1	1,420	167.3 ± 0.7	1,416	20.5 ± 0.1	847	178.5 ± 1.0	845

dents at our University from 1955 to 1995 was 20.9 (2.9 kg/m^2 (mean ± SEM, n = 7,771) and 20.7 (2.1 kg/m^2 (n = 3,304), respectively. Subjects were divided into 5 groups according to BMI: lean (L) group, < 17 kg/m^2; lean-normal (LN) group, ≥ 17 and < 19; normal (N) group, ≥ 19 and < 23; normal-overweight (NO) group, ≥ 23 and < 25; and overweight (O) group, ≥ 25 kg/m^2.

Serum TC was analyzed on a Hitachi 7350 automated clinical chemistry analyzer (Tokyo, Japan) using reagents supplied by the manufacture (Auto-L-TCHO, Mizuho Medy, Co., Saga, Japan). All samples were measured in one day. The laboratory was standardized for lipid measurements through the Japanese Control Survey Program (Japanese Association of Medical Technologist). The coefficient of variation (CV) of the serum TC assay was < 2.5%. Normal TC levels imply a serum concentration of < 200 mg/dl, as this value has been recommended as optimal by the Japan Atherosclerosis Society [12].

Statistical Analysis. Data are expressed as mean ± SEM. Differences between groups were analyzed for statistical significance using a one-way analysis of variance followed by a post-hoc Fisher test or analysis of variance when appropriate. A P value of < 0.05 was considered to denote the presence of a statistically significant difference.

Results

Effect of blood-sample preservation on serum TC. The correlations between original and present values of measured serum TC are shown in Fig. 1. The 2 sets of serum TC measurements showed a significant correlation irrespective of the storage period, implying that storage of blood samples at −80 °C for 1, 3, 5, 6, 8, or 10 years dose not affect serum TC measurement.

BMI and serum TC in all subjects. Table 2 shows body height, BMI, and serum TC levels for each year for males and females. There was no significant difference in body height during the 10-year period. The BMI values for both males and females in 1998 were significantly lower than those in 1993. However, serum TC levels in 1998 were significantly higher than in 1993 in both sexes. In male subjects, the proportion of subjects with normal BMI gradually decreased from 1989 to 1998 (Fig. 2-top), and that of the normal TC population among total subjects also decreased during this period (Fig. 2-bottom). With regard to female students, the

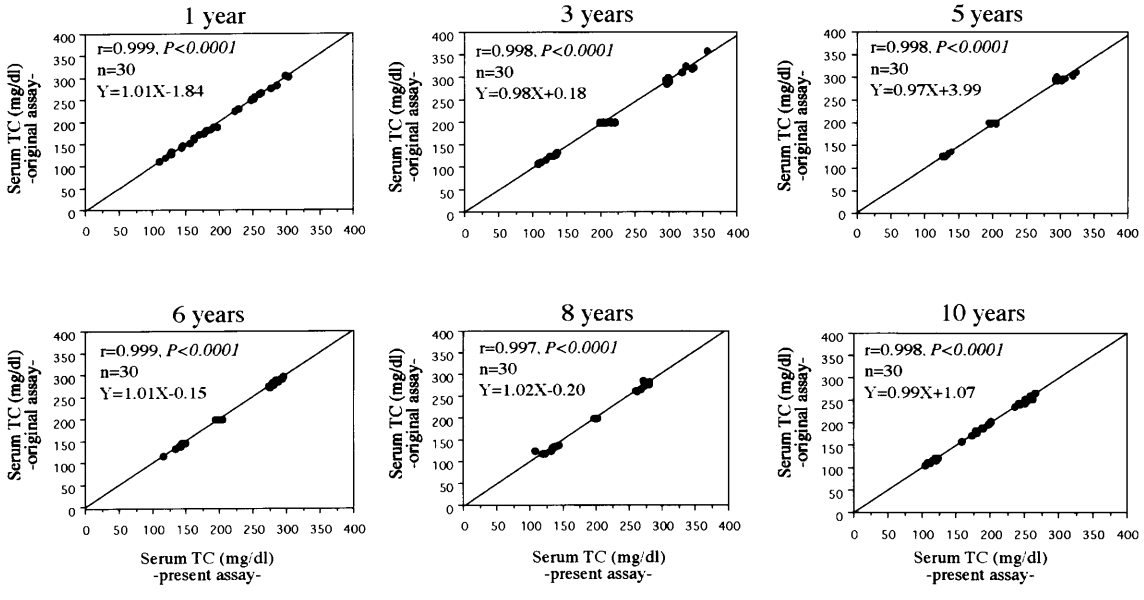

Fig. 1 Correlation of serum total cholesterol (TC) concentrations between original and present values. Original values of TC measured by different assay kits were converted using the calculation formula described in Material and Methods and were compared with present values measured by a single assay using stored serum samples. The duration of the storage of serum samples at −80 °C is indicated above each graph.

proportion of students with normal BMI and normal serum TC decreased only from 1993 to 1998 (Fig. 2).

The correlation between BMI and serum TC levels was weak ($r = 0.21$) but significant ($P < 0.001$) for all male subjects ($n = 3,708$, Fig. 3), while there was no relationship between the 2 parameters in females ($r = 0.091$, $n = 2,051$, Fig. 3). The correlation coefficients between the 2 parameters in 1989, 1993, and 1998 in males were 0.229, 0.242, and 0.178, respectively, while those in females were 0.107, 0.113, and 0.075, respectively. In both male and female subjects, we observed a tendency toward a lower correlation between BMI and serum TC in 1998 than in other entrance years. To examine the relationship between the degree of obesity and serum TC levels, we analyzed the relationship between serum TC and various predefined sets of BMI

Table 2 Body height, BMI, and serum total cholesterol (TC) concentrations of all subjects that enrolled at Okayama University from 1989-1998

Entrance	Males			Females		
Year	1989	1993	1998	1989	1993	1998
Number	1,102	1,155	1,399	611	626	807
Height (cm)	171 ± 0.2	171 ± 0.2	171 ± 0.2	158 ± 0.2	158 ± 0.2	158 ± 0.2
BMI (kg/m^2)	21.2 ± 0.08	21.5 ± 0.08*	21.2 ± 0.08#	20.7 ± 0.09	20.8 ± 0.10	20.5 ± 0.09#
Serum TC (mg/dl)	158.7 ± 0.8	162.6 ± 0.8†	165.0 ± 0.7†#	172.0 ± 1.1	171.7 ± 1.1	175.6 ± 1.0*#

*$P < 0.05$ and †$P < 0.001$ *vs.* sex-matched data of 1989; #$P < 0.05$ *vs.* sex-matched data of 1993.

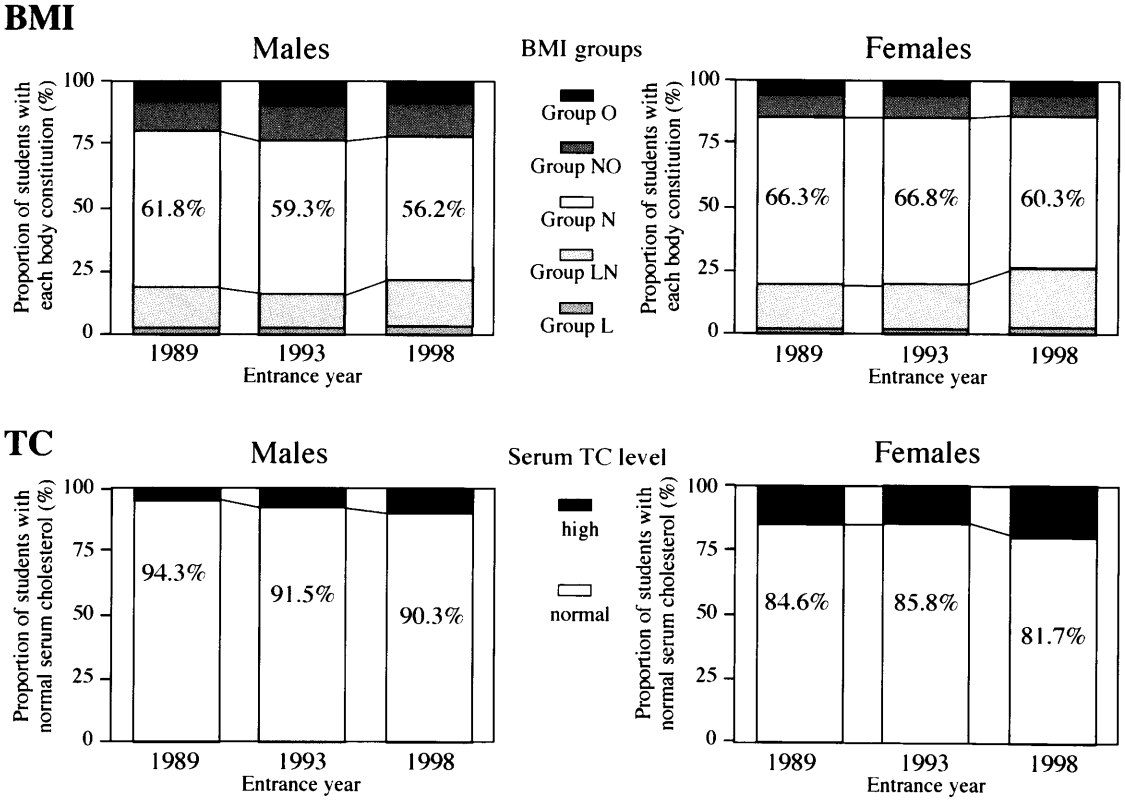

Fig. 2 Serial changes in the proportion of students with various BMI values (top) and normal TC (bottom) in males and females. Criteria used for the definition of normal TC and each BMI group are described in Material and Methods.

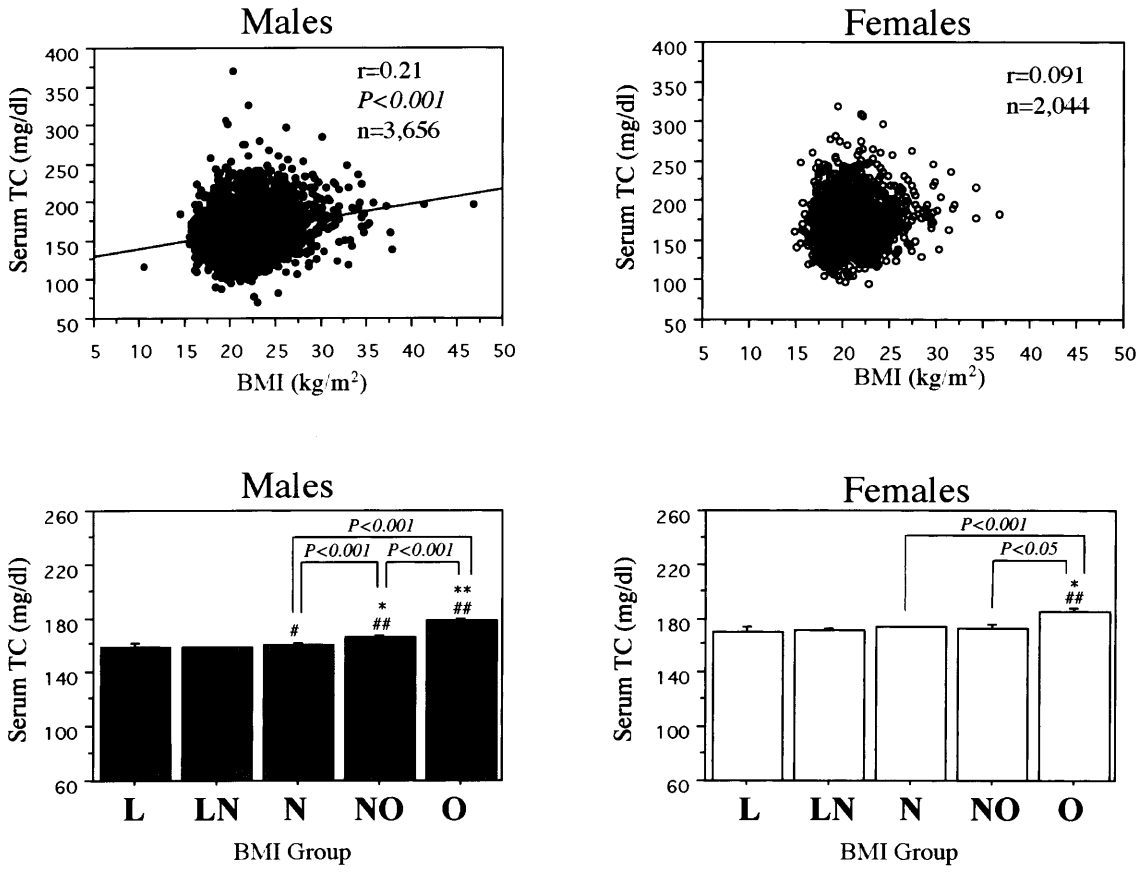

Fig. 3 Correlation between BMI and serum total cholesterol (TC) concentrations (top) and serum TC levels and BMI groups (bottom) in males and females (data are mean ± SEM). Criteria used for the definition of each BMI group are described in Material and Methods.

(Fig. 3). Serum TC concentrations tended to increase with progressive rises in BMI, implying that serum TC concentrations tend to increase according to the degree of obesity. In addition, this tendency appears to be more apparent in males than females (Fig. 3).

Serial changes in serum TC among the BMI groups. We also examined the incidence of hypercholesterolemia in each BMI group for each entrance year (Fig. 4). In 1989, the highest incidence of hyperlipidemia was noted in the O group. Interestingly, the high incidence of hyperlipidemia was recognized not only in the obese (NO + O) group, but also in non-obese (LN + N) groups in both 1993 and 1998. This tendency was more remarkable in females than in males. In addition, even in normal BMI subjects (N group), the incidence of hypercholesterolemia tended to increase in recent entrance years in both sexes (male, 4.6, 7.0, and 7.4%; female, 14.6, 14.6, and 19.0% in 1989, 1993, and 1998, respectively). Finally, we compared the serum TC levels among the three BMI-graded groups (L + LN, N, and NO + O group) in 1989, 1993, and 1998 (Fig. 5). In the lean (L + LN) group, the serum TC increased from 1989 to 1998 in males, but there was no significant change in females in the 3 years (Fig. 5-left). Although the proportion of subjects in the normal BMI (N) group was similar in the 3 years, the serum TC levels in males significantly and gradually increased from 1989 to 1998, while those in females increased in 1998 (Fig. 5-middle). Contrary to the normal BMI group, there were no significant differences in serum TC levels in the overweight (NO + O) group among the 3 years in both males and females (Fig. 5, right).

Discussion

The present study demonstrated that serum TC levels

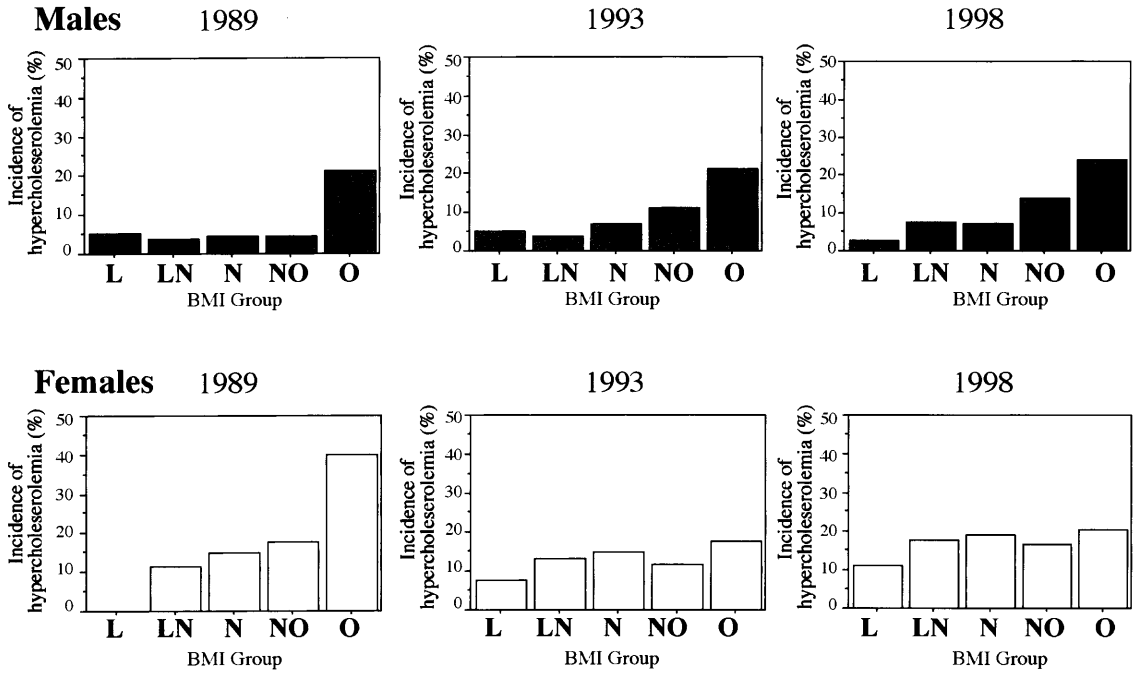

Fig. 4 Proportion of subjects with hypercholesterolemia according to BMI in 1989–1998. Criteria used for the definition of hypercholesterolemia and each BMI group are described in Material and Methods.

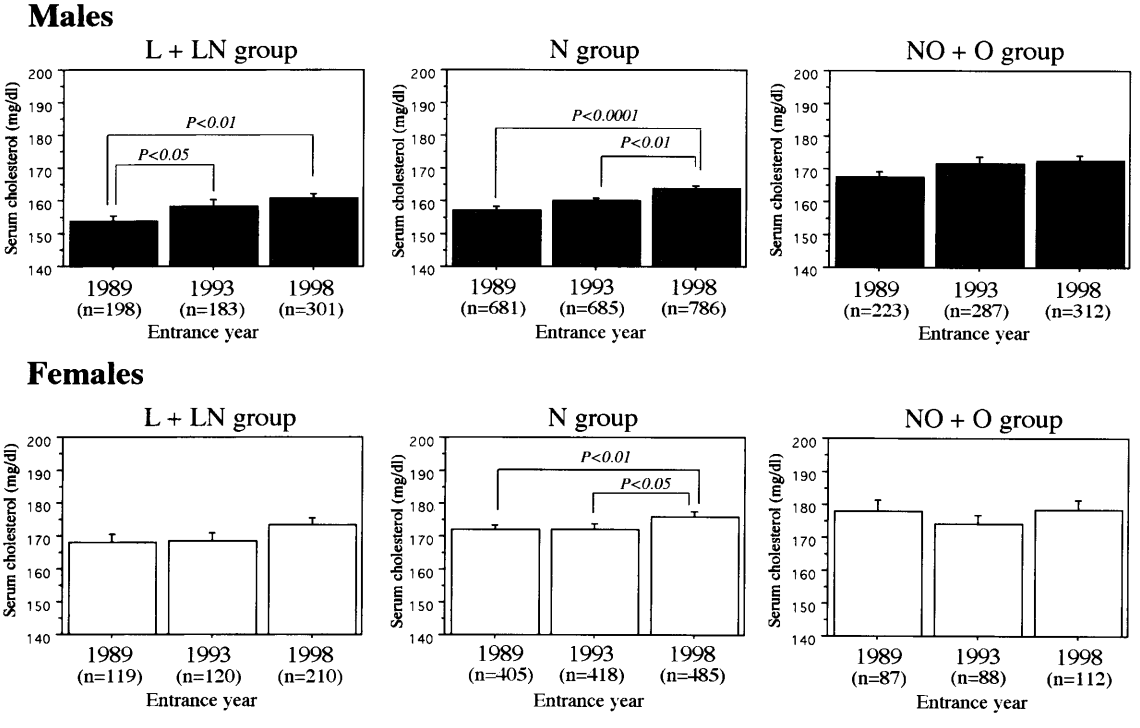

Fig. 5 Serial changes in serum total cholesterol (TC) concentrations among BMI-graded groups. Criteria used for the definition of each BMI are described in Material and Methods.

and the incidence of hypercholesterolemia increased from 1989–1998 in newly enrolled students at our university. Interestingly, this result was also confirmed among BMI-matched non-obese (BMI, ≥ 19 and < 23) subjects. These findings imply that the recent trend of hypercholesterolemia may not necessarily depend on the presence of obesity. Since this trend could probably be applied to Japanese young adolescents at large, it becomes important to ascertain the background of this phenomenon in order to protect this population against hypercholesterolemia.

In the first part of the study, we examined the effects of serum storage on TC levels. Since 1989, the parts of the serum samples remaining after the matriculation health examination had been carefully preserved at $-80\,^{\circ}C$ at the Health and Medical Center. The main purpose of storage is to utilize the preserved serum for future epidemiological studies. Previous studies have shown that TC measurements remain stable even when stored serum samples are used for analysis [13, 14]. Our preliminary study showed that 1 to 10-year storage of sera *per se* had no effect on the stability of the TC measurements, as long as the serum samples were stored at $-80\,^{\circ}C$ without liquefaction. Shih *et al.* [15] have indicated that there is a small but consistent decrease in TC concentrations in response to storage of sera at $-70\,^{\circ}C$. The different results may depend on the condition of storage or the method of TC measurement.

The BMI has generally been used as an index of obesity [16, 17]. In adults, a BMI cutoff value of 30 kg/m^2 has been used to define obesity [17, 18]. The obese population in Western countries is reported to constitute 15–25% of the general population [19], while constituting less than 2–3% in Japan [20]. Even when using the recent definition of obesity of BMI $> 25\ kg/m^2$ [21], the incidence of obesity in Japanese males and females is only 21% and 7%, respectively [8], which is still lower than that reported in Western countries. Despite these figures, our previous study [22] showed a high morbidity of 53.9% and 40.9% in our male and female subjects, respectively (unpublished observation). Although the correlation between obesity and increased mortality and morbidity is well established, previous and our present findings raise an important issue, *i.e.* how do we assess body weight in young (not only overweight but also nonobese) adolescents with respect to the future development of LRD?

Investigation of the changes in body constitution extending over a long period is both epidemically and socioeconomically important, since longevity and morbidity are related to height and body size [23]. Between 1955 and 1995, body height (males, 164.8 ± 0.22 to 171.4 ± 0.15; females, 153.3 ± 0.29 to 158.1 ± 0.19 cm) and body weight (males, 54.7 ± 0.23 to 63.4 ± 0.25; females, 49.1 ± 0.34 to 52.0 ± 0.26 kg), respectively, have markedly increased among students entering our university. These changes have also been accompanied by a steady and progressive increase in the proportion of overweight and/or normal-overweight males entering the university in successive enrollment years during the 40-year period, which has been associated with a fall in the proportion of males with normal BMI [7]. For the period between 1989–1998, both the BMI and the proportion of male students with normal BMI did not change from 1993 to 1998. Excluding genetic and gender factors, overweight was considered to be a primary cause of hypercholesterolemia. Indeed, BMI has correlated weakly but significantly with serum TC in male subjects. Nevertheless, our present study indicates that both serum TC levels and the proportion of subjects with high serum TC has increased among adolescents not classified as obese. Indeed, our study showed that the serum TC levels did not increase among overweight subjects in both sexes from 1989 to 1998, although they did increase among the normal BMI subjects. In this regard, we suggest that there may be a large number of Japanese subjects with "masked obesity", which is not defined as obesity but is associated with LRD due to an accumulation of visceral fat [8]. The underlying cause of the increased proportion of individuals with "masked obesity" is probably related to a westernization of diet and low levels of exercise in the young generation. For example, we have reported the presence of a high proportion of obese and non-obese male freshmen among our university students with abnormally high serum concentrations of alanine aminotransferase (ALT) due to fatty liver [24]. Fortunately, this is a transient phenomenon, since high serum ALT levels were found to return to normal levels 2–3 months later when BMI or percent body fat subsequently decreased [24]. Generally speaking, the Japanese university entrance examination, like those in many other countries, is difficult to conduct, and there is a tendency among high school students to stop physical activity and exercise during preparation for the high school certificate. It is possible that the peculiar circumstances related to the entrance examination in Japanese

universities may also predispose students to the development of hyperlipidemia or fatty liver due to a transient increase in body weight.

In conclusion, we have demonstrated in the present study a significant rise in the proportion of non-obese university students with high serum TC concentrations from 1989–1998. Our findings suggest that the spread of LRD may occur among young Japanese adolescents irrespective of an increase in the obese population. We need to encourage young adolescents to pursue a healthier life-style to protect them against LRD. For this purpose, we are currently investigating the life-style of university students.

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