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Circadian rhythm of serum concentration of small dense low-density lipoprotein cholesterol

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Abstract

Background: The atherogenicity of small dense low-density lipoprotein (sdLDL) has been reported and recently a new easy-handling method for measuring serum concentration of sdLDL cholesterol (sdLDLC) has been developed. Using this method, we observed the circadian rhythm of sdLDLC to determine the adequacy of fasting measurement of it and to seek the modulator of the atherogenic lipoprotein.
Methods: Study population was consisted of 20 healthy volunteers (10 women and 10 men, mean age 28 y). They had 3 meals per day and blood samples were taken before and 2 h after every meal and next morning. Serum concentrations of sdLDLC and other valuables including triglyceride (TG) and remnant-like particles cholesterol (RLPC) were determined.
Results: Serum concentration of sdLDLC had a unique circadian rhythm that was highest before breakfast (fasting status), decreased after each meal, hit the bottom after dinner and then increased during at night. Fasting sdLDLC was highly correlated with TG levels. The sum of the 6 TG values during a day (i.e., average TG level) had higher correlation coefficient with sdLDLC than fasting TG or fasting RLPC.
Conclusions: From the observation of the unique circadian rhythm, measuring sdLDLC at fasting status is exactly reasonable because it never underestimate the risk of atherosclerotic diseases. Measuring sdLDLC can also be used as a marker for average TG levels regardless of the existence of postprandial hyperlipidemia.

Keywords: Small dense LDL cholesterol; Circadian rhythm; Postprandial hyperlipidemia; Triglyceride; Atherosclerosis

1. Introduction

Low-density lipoprotein (LDL) is known as an atherogenic lipoprotein and especially smaller and denser fraction of it is more atherogenic [1–8]. Many epidemiological and pathological studies have reported the close relationship between the cause of coronary artery disease and hypercholesterolemia especially in the fraction of small dense LDL (sdLDL). Many studies on sdLDL have been based on the measurement of the particle size of sdLDL or the ratio of sdLDL to total LDL mainly obtained by gradient gel electrophoresis or chromatography [9]. Nuclear magnetic resonance (NMR) spectroscopy and ultracentralization have been used for quantification of sdLDL, however, it is difficult to measure a lot of samples by those procedures because it takes much time to measure one [10,11]. The atherogenicity of sdLDL has been explained by many aspects and one of them is its cholesterol content which is more likely to be brought to

Abbreviations: LDL, low-density lipoprotein; LDLC, low-density lipoprotein cholesterol; sdLDL, small dense low-density lipoprotein; sdLDLC, small dense low-density lipoprotein cholesterol; TG, triglyceride; RLP, remnant-like particle; RLPC, remnant-like particles-cholesterol; RLPTG, remnant-like particles-triglyceride; PPHL, postprandial hyperlipidemia; HDLC, high-density lipoprotein cholesterol; TC, total cholesterol; meanTG, mean of the 6 TG values during a day; CAD, coronary artery disease; OFTT, oral fat tolerance test.

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Table 1
Characteristics of the subjects

Gender (M/F)	10/10	
Age (yrs)	28.1±1.1	(21–41)
Body mass index	21.0±0.5	(17.7–26.5)
Fasting plasma glucose (mmol/L)	4.88±0.07	(4.44–5.44)
Fasting insulin (pmol/L)	36.6±3.1	(18.0–72.0)
HbA1c(%)	4.6±0.1	(4.2–5.1)
Total cholesterol (mmol/L)	4.94±0.15	(3.42–6.15)
Triglyceride (mmol/L)	1.09±0.15	(0.32–2.01)
HDL cholesterol (mmol/L)	1.73±0.09	(1.08–2.38)
LDL cholesterol (mmol/L)	2.91±0.14	(2.02–4.42)
RLP-cholesterol (mmol/L)	0.18±0.02	(0.08–0.31)
RLP-triglyceride (mmol/L)	0.18±0.04	(0.03–0.49)

Data were shown as mean±SE.
HbA1c = glycohacmoglobin A1c.
HDL = high-density lipoprotein.
LDL = low-density lipoprotein.
RLP = remnant-like particles.

atherosclerotic plaques [12]. Therefore, we should determine the amount of sdLDL cholesterol (sdLDLC) rather than the particle size. Recently, a new method developed to measure the serum concentration of sdLDLC directly [13,14]. Using this method, some evidence have been published [14,15]. Higher serum sdLDLC concentrations were observed in patients with coronary artery diseases than healthy people. In patients with hypertriglyceridemia, sdLDLC were also higher than normolipidemic individuals [14]. However, there were no previous observations on circadian rhythm of serum concentration of sdLDLC or the determination on the factors that modulate serum sdLDLC concentration. Using this method, we observed the circadian rhythm of serum sdLDLC concentration and determined the relationship between sdLDLC and other parameters including serum concentrations of triglyceride (TG) and remnant-like particles-cholesterol (RLPC) as markers of postprandial hyperlipidemia (PPHL).

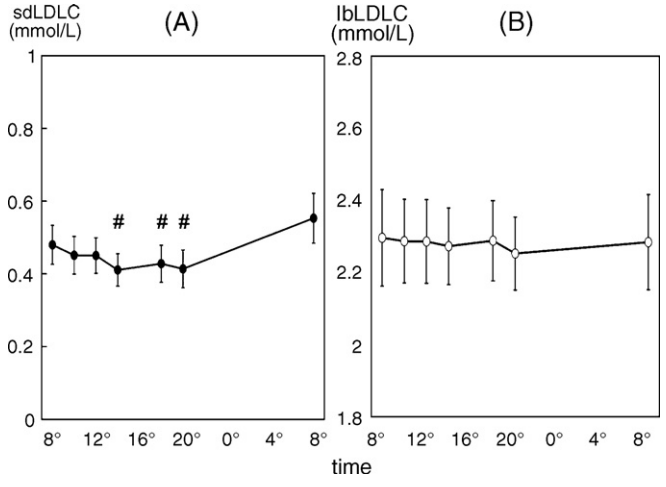

Fig. 1. Circadian rhythm of serum concentration of small dense LDL-cholesterol (sdLDLC) (A) and calculated large buoyant low-density lipoprotein-cholesterol (lbLDLC) (B) in all subjects. While the value of lbLDLC did not change significantly during the observation period, the value of sdLDLC was highest before breakfast that was fasting status, decreased after each meal, hit the bottom after dinner and then increased during a night. #: The changes from baseline were statistically significant by p value<0.05.

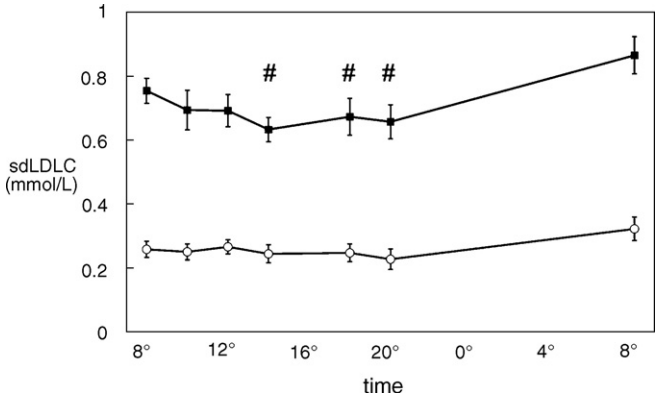

Fig. 2. Circadian rhythm of serum concentration of small dense LDL-cholesterol (sdLDLC). White circles showed the mean values of sdLDLC in individuals with higher fasting sdLDLC level and black circles showed those with lower fasting sdLDLC level. In those with lower fasting sdLDLC level, the values of sdLDLC were almost stable during a day, in contrast, in those with higher fasting sdLDLC level, the value of sdLDLC was highest before breakfast that was fasting status, decreased after each meal, hit the bottom after dinner and then increased during a night. #: The changes from baseline were statistically significant by p value<0.05.

2. Materials and methods

This study conformed to the Helsinki Declaration and all study protocols were approved by the ethical committee of Tokyo Medical and Dental University. The study population was consisted of 20 healthy volunteers (10 men and 10 women, aged 28 y). The characteristics of the subjects are shown in Table 1. No participants consumed lots of alcohol and there were no smokers and taking no medicine. After an overnight fasting, all the participants performed the program. They had 3 meals a day at 8 a.m., 12 noon and 6 p.m. Blood samples were obtained from all the subjects 7 times in total: (before and 2 h after each meal and the next morning at 8 a.m. before breakfast). The participants took between 500 and 800 Kcal consisted of 50–60% of carbohydrates, 20–30% of fat and about 20% of protein per each meal. Each participant selected each meal as they liked. Nothing except water was allowed to intake during the observation. Serum concentrations of insulin [16], glucose [17], total cholesterol (TC) [18], TG [19], high-density lipoprotein cholesterol (HDLC) [20], LDLC [13,21], RLPC [22,23], remnant-like particles triglyceride (RLPTG) [22,23], and sdLDLC [13,14] were measured.

The measured values are presented as the mean±standard error (SE). For statistical analysis, paired t-tests were used for each time point and between time points and repeated measures analysis of variance (ANOVA) was used to examine chronological changes. Correlation coefficients were determined between sdLDLC and other variables using Spearman's correlation test. In all cases, values of $p<0.05$ were considered statistically significant.

3. Results

In the circadian rhythm of all the subjects, serum concentration of sdLDLC was the highest before breakfast (at fasting), decreased after each meal, hit the bottom at 2 h after dinner and then increased to the top during a night (Fig. 1−A). When the subjects were divided into 2 groups by their sdLDLC concentration at fasting, in the group of lower sdLDLC level, their sdLDLC concentration did not change during the study period, while in the group of higher sdLDLC level, their sdLDLC concentrations were the highest at fasting and the lowest after