classes were calculated based on the particle diameter as follows: (sum of particle diameter x lipid concentration in each fraction)/(sum of lipid concentration of the fractions of interest). Ultracentrifugation fractionation followed by determination of lipid contents was performed in Pt. 1, 2 and 5 (Table I in data supplement). For some FLD sera, representative fractions were collected, concentrated by Vivaspin (MWCO=3,000, Sartorius, Weender Landstr., Germany) and subjected to determine apolipoprotein concentrations by Milliplex MAP Human Apolipoprotein Panel kit (Millipore, Billerica, MA) using BioPlex apparatus (BioRad, Hercules, CA).

Preparation of recombinant LCAT and *in vitro* incubation with patient's serum

Human *LCAT* gene was transduced into human preadipocytes by retroviral vector as described previously[1]. The resulting cells were seeded into T225 flask and grown to confluency in MesenPRO medium (Life Technologies, Carlsbad, CA). The medium was changed to 30 ml of OPTI MEM I (Life Technologies) and the cells were further incubated for seven days to collect culture supernatant. The culture supernatant was concentrated to one-fiftieth of the original volume by Amicon Ultra (MWCO=50 kDa, Millipore)[4]. LCAT concentration was titrated by immunoblotting using commercially available human rLCAT (Roar Biomedical, Inc., Calverton, NY) as standard. After mixing with rLCAT-containing culture supernatant at the ratio of 29:71 (v/v), each patient's serum was incubated at 37 °C for 24 hr (final concentration of rLCAT was 6 μg/ml), and subjected to lipoprotein analysis. Culture supernatant of human preadipocytes without gene transduction was used as control.

Statistical analysis

Data are presented as means ± S.D. Comparisons were assessed for significant differences by paired Student's *t*-test, or by ANOVA followed by Tukey test, where appropriate. SPSS software was used for statistical analyses. In all cases, P values of less than 0.05 were considered significant.

REFERENCES:

1. Kuroda M, Aoyagi Y, Asada S, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Machida K, Matsumoto F, Satoh K, Aso M, Saito Y. Ceiling culture-derived

proliferative adipocytes are a possible delivery vehicle for enzyme replacement therapy in lecithin: cholesterol acyltransferase deficiency. *The Open Gene Ther J* 2011;4:1-10

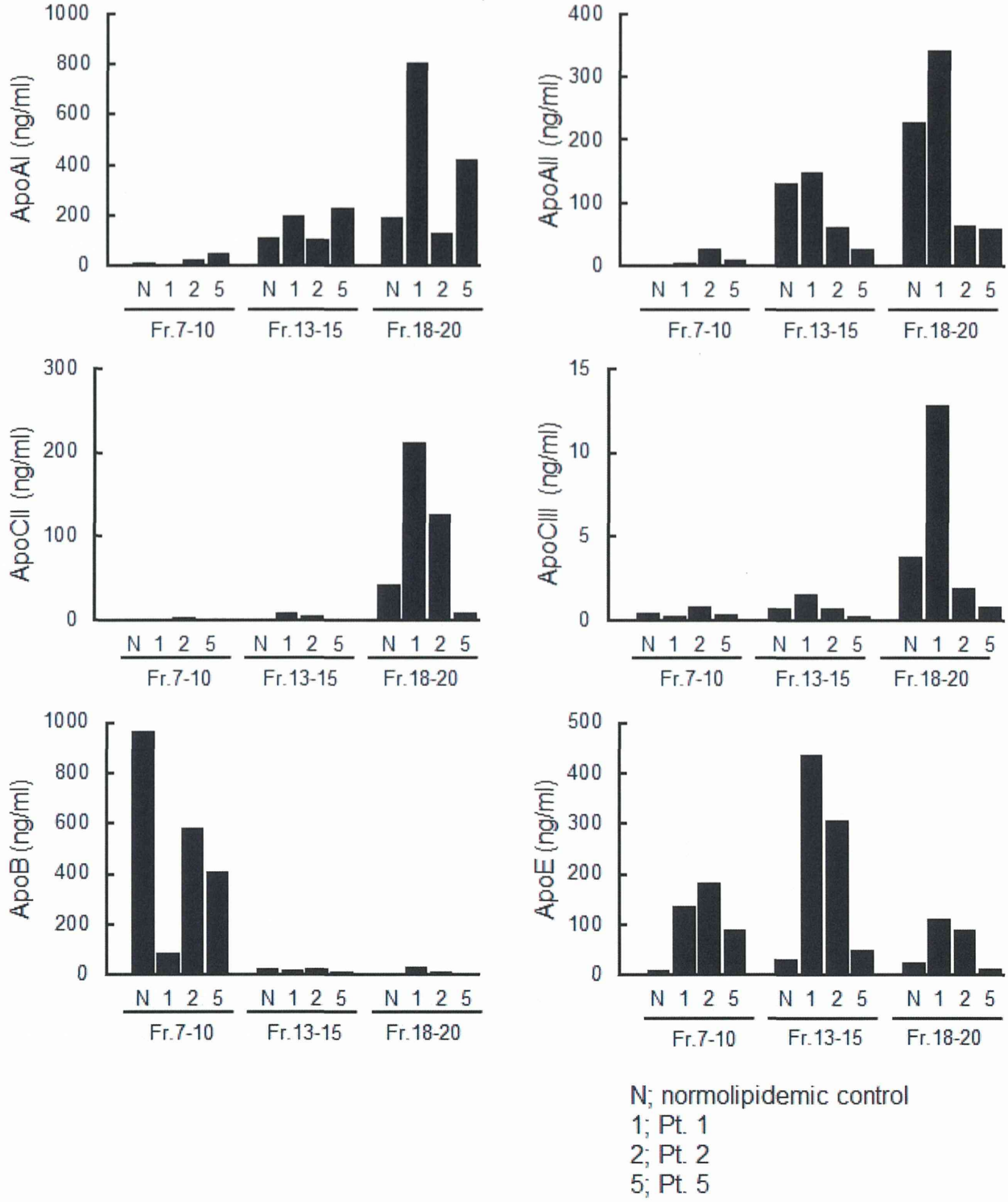
2. Okazaki M, Usui S, Ishigami M, Sakai N, Nakamura T, Matsuzawa Y, Yamashita S. Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in high-performance liquid chromatography. *Arterioscler Thromb Vasc Biol* 2005;25:578-584
3. Usui S, Hara Y, Hosaki S, Okazaki M. A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. *J Lipid Res* 2002;43:805-814
4. Asada A, Kuroda M, Aoyagi Y, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Aso M, Saito Y. Disturbed apolipoprotein A-I-containing lipoproteins in fish-eye disease are improved by the lecithin:cholesterol acyltransferase produced by gene-transduced adipocytes *in vitro*. *Mol Genet Metab* 2010;102:229-231

Supplement Table I

		Pt. 1	Pt. 2	Pt. 5
HDL				
	Total cholesterol (mg/dl)	18.6	12.0	10.8
	Triglyceride (mg/dl)	3.6	3.0	4.2
	Phospholipid (mg/dl)	65.4	44.4	42.0
LDL				
	Total cholesterol (mg/dl)	62.4	99.0	39.6
	Triglyceride (mg/dl)	77.4	151.2	61.8
	Phospholipid (mg/dl)	106.2	157.8	73.2
VLDL				
	Total cholesterol (mg/dl)	62.0	51.0	9.6
	Triglyceride (mg/dl)	190.0	157.8	36.0
	Phospholipid (mg/dl)	103.4	81.8	21.8

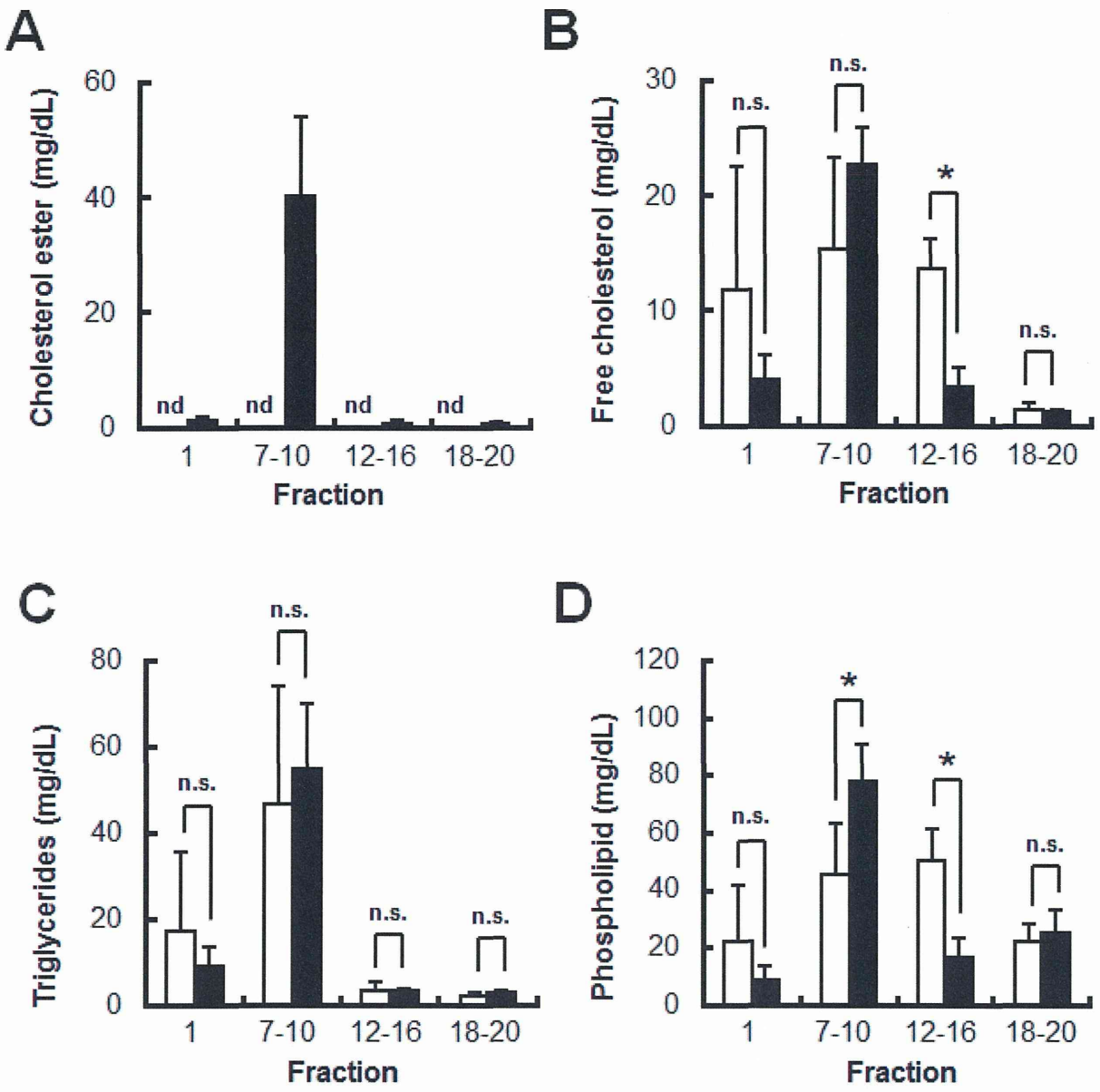
Supplement Table I. Ultracentrifugation analysis of lipoprotein in FLD patients.
Patients' sera were subjected to ultracentrifugation fractionation, followed by determination of lipid concentration.

Supplement Figure I



Supplement Figure I. Apolipoprotein contents in lipoproteins in FLD patients.
Lipoprotein subfractions were collected and concentrated (MWCO=3,000). Apolipoprotein concentrations in the concentrated samples were determined by ELISA. N; normolipidemic control, 1; Pt. 1, 2; Pt. 2, 5; Pt. 5.

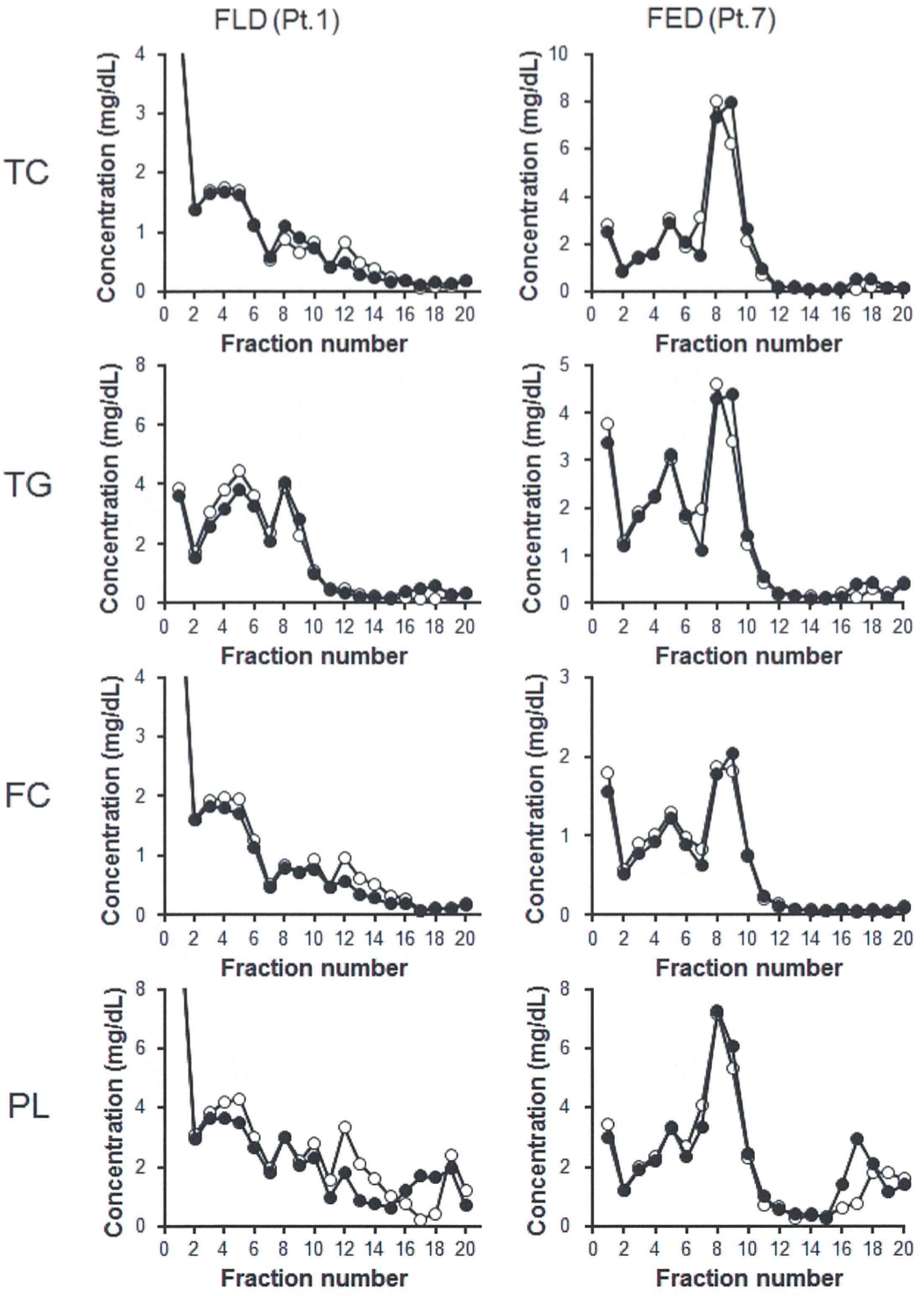
Supplement Figure II



Supplement Figure II. Comparison of lipid concentrations of lipoproteins between FLD and FED.

According to the distribution of lipoproteins shown in Figure 1, CE (panel A), FC (panel B), TG (panel C), and PL (panel D) concentrations in Fr. 1, Fr. 7-10, Fr. 12-16 and Fr. 18-20 were compared between FLD patients (open column, n=5) and FED patients (closed column, n=4). nd, not detected, *p<0.05.

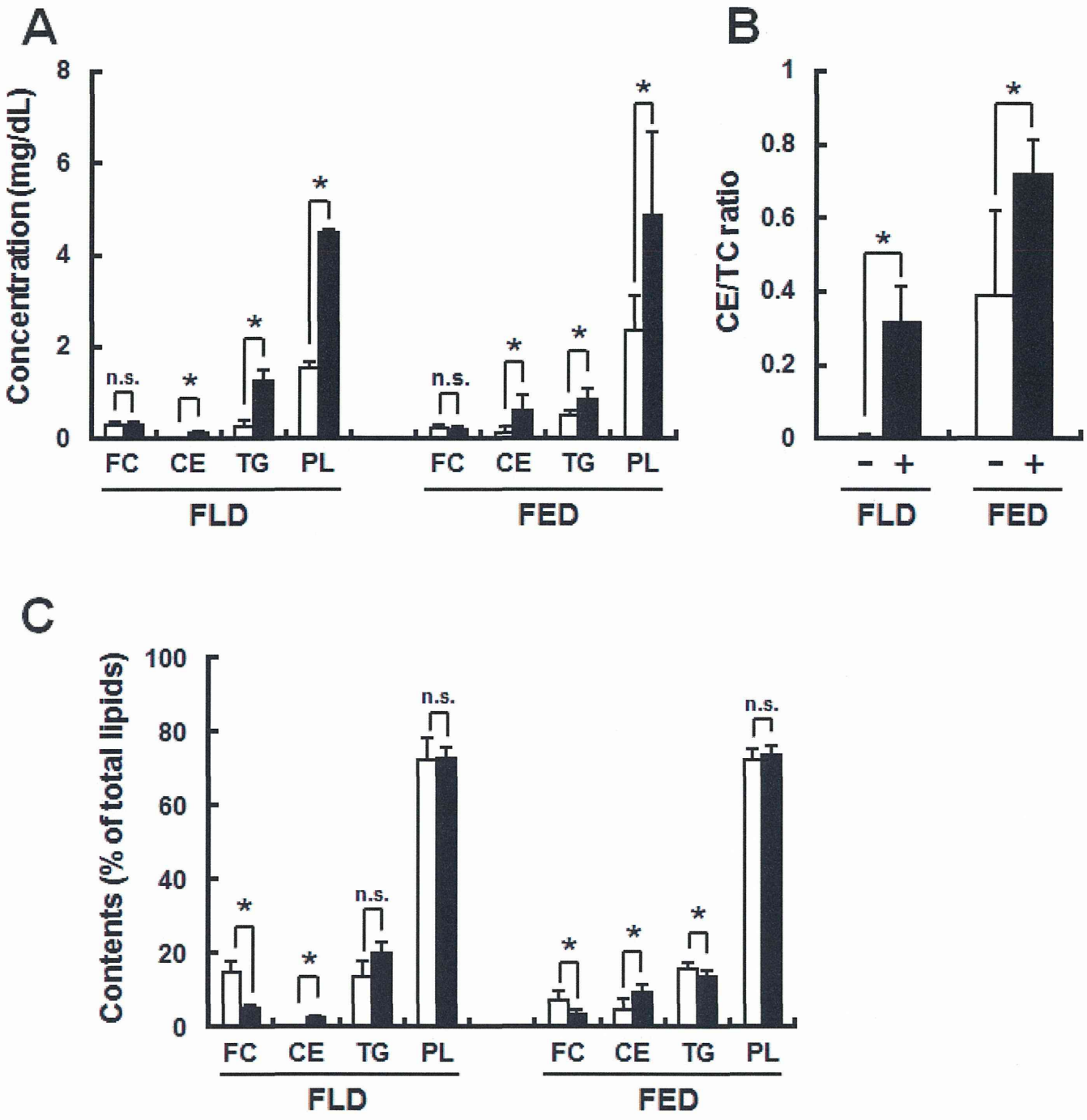
Supplement Figure III



Supplement Figure III. Response to rLCAT *in vitro*

After incubation with culture supernatant of human *LCAT* gene-transduced preadipocytes (closed circle) and un-transduced control (open circle), GFC analyses with simultaneous determination of TC, TG, FC, and PL concentrations were performed. Representative results of FLD (Pt. 1) and FED (Pt. 7) sera were shown.

Supplement Figure IV



Supplement Figure IV. *In vitro* rLCAT incubation ameliorated HDL composition in FLD and FED.

After *in vitro* rLCAT incubation and subsequent GFC analyses of lipoproteins shown in Fig. S2, changes of core HDL fractions (Fr. 16-18) were analyzed in FLD (n=4) and FED (n=4) sera (A-C). Lipid concentrations (A), CE/TC ratio (B), and lipid composition (C) were compared between rLCAT containing culture media (closed bar) and the culture media without rLCAT (open bar). *p<0.05.

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Association of lifestyle-related factors with circadian onset patterns of acute myocardial infarction: a prospective observational study in Japan

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ABSTRACT

Objective: The onset of acute myocardial infarction (AMI) shows characteristic circadian variations involving a definite morning peak and a less-defined night-time peak. However, the factors influencing the circadian patterns of AMI onset and their influence on morning and night-time peaks have not been fully elucidated.

Design, setting and participants: An analysis of patients registered between 1998 and 2008 in the Osaka Acute Coronary Insufficiency Study, which is a prospective, multicentre observational study of patients with AMI in the Osaka region of Japan. The present study included 7755 consecutive patients with a known time of AMI onset.

Main outcomes and measures: A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI had uniform, unimodal or bimodal distribution, and the likelihood ratio test was then used to select the best circadian pattern among them. The hierarchical likelihood ratio test was used to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier method was used to estimate survival curves of 1-year mortality according to AMI onset time.

Results: The overall population had a bimodal circadian pattern of AMI onset characterised by a high and sharp morning peak and a lower and less-defined night-time peak (bimodal $p<0.001$). Although several lifestyle-related factors had a statistically significant association with the circadian patterns of AMI onset, serum triglyceride levels had the most prominent association with the circadian patterns of AMI onset. Patients with triglyceride ≥150 mg/dL on admission had only one morning peak in the circadian pattern of AMI onset during weekdays, with no peaks detected on weekends, whereas all other subgroups had two peaks throughout the week.

Conclusions: The circadian pattern of AMI onset was characterised by bimodality. Notably, several lifestyle-related factors, particularly serum triglyceride levels, had a strong relation with the circadian pattern of AMI onset.

Strengths and limitations of this study

- We comprehensively analysed the circadian patterns of acute myocardial infarction (AMI) onset in a large, multicentre cohort of patients in relation to patient characteristics, lifestyle factors and the day of the week.
- A mixture of two von Mises distributions revealed that the circadian pattern of AMI onset exhibited bimodality.
- Several lifestyle-related factors were shown to be associated with the circadian patterns of AMI onset, depending on the day of the week. In particular, it was demonstrated that elevated serum triglyceride levels on admission accentuated morning peak of AMI onset during weekdays.
- Participants were limited to those who were hospitalised for AMI.
- Laboratory data were evaluated on admission.

Trial registration number: UMIN000004575.

INTRODUCTION

Onset patterns of acute myocardial infarction (AMI) exhibit circadian variation which is characterised by an increased frequency in the morning and a secondary peak incidence at night-time.[1] Several studies have confirmed that AMI onset exhibits a bimodal circadian pattern, with peaks occurring in the morning[2–4] and night-time hours.[1 4–7] However, it is not well understood what factors, particularly lifestyle-related factors, influence the circadian patterns of AMI. Moreover, although these patterns appear to vary according to the day of the week,[8] it is unclear how the circadian patterns

of AMI onset vary throughout the week, particularly, in association with socioeconomic factors.

As AMI and subsequent ischaemic heart failure is the leading cause of death in developed and developing countries, primary prevention of AMI is a major healthcare issue worldwide. Accordingly, identifying potential factors influencing the circadian pattern of AMI may help in the clinical management of patients to prevent the onset of AMI.

In the present study, we comprehensively analysed the circadian patterns of AMI onset in a large, multicentre cohort of patients in relation to patient characteristics, lifestyle factors and the day of the week.

METHODS

OACIS registry and study participants

The Organisation to Assess Strategies for Ischaemic Syndromes (OACIS) is a prospective, multicentre observational study collecting demographic, procedural, biological and outcome data as well as blood samples from patients with AMI hospitalised at 25 collaborating hospitals from the Osaka region of Japan (UMIN-Clinical Trial Registry ID: UMIN000004575; see online supplementary appendix).[9 10] A diagnosis of AMI was made if the patient fulfilled at least two of the following three criteria: (1) history of central chest pressure, pain or tightness lasting 30 min, (2) typical ECG changes (ie, ST-segment elevation ≥0.1 mV in one standard limb lead or two precordial leads, ST-segment depression ≥0.1 mV in two leads, abnormal Q-waves or T-wave inversion in two leads) and (3) an increase in serum creatine kinase levels two times the upper normal limit in each hospital. All the collaborating hospitals were encouraged to enrol consecutive patients with AMI.

We prospectively collected data with the help of research cardiologists and trained research nurses using a specific reporting form, and the following variables were extracted from the OACIS registry database: age, gender, working status, body mass index, coronary risk factors (diabetes, hypertension, dyslipidaemia, smoking, drinking, previous MI, multivessel disease and collateral circulation), clinical presentation on admission (KILLIP classification, initial TIMI flow and ST-elevation MI (STEMI)), coronary angiography data, reperfusion therapy, laboratory data on admission (glycated haemoglobin (HbA1c), total cholesterol, low-density (LDL) and high-density lipoprotein (HDL) cholesterol, triglyceride and estimated glomerular filtration rate) and medications at discharge (RAS inhibitors, β-blocker, calcium channel blocker, statin, antiplatelet agent and diuretics). Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dL, HbA1c ≥6.5% or a history of antidiabetic therapy. Hypertension was defined as a history of systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or antihypertensive therapy. Dyslipidaemia was defined as fasting total cholesterol ≥220 mg/dL, LDL cholesterol ≥140 mg/dL, HDL cholesterol ≤40 mg/dL, fasting triglycerides ≥150 mg/dL or lipid-lowering therapy.

The study protocol has been approved by the ethics committee of each participating hospital. All in-hospital data were obtained after written informed consent had been received and were then transmitted to the data collection centre at the Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan, for processing and analysis. The corresponding authors had full access to and validated all data in the study.

In the present study, we analysed 7755 patients with AMI whose time of AMI onset was definitely known among the 8603 consecutive patients registered in the OACIS registry between 1998 and 2008. Patients' baseline characteristics are presented in table 1.

Statistical analysis

Continuous variables were summarised as quartiles and were compared using the Wilcoxon rank-sum test for two-group comparisons, and the Kruskal-Wallis test for four-group comparisons. Categorical variables were presented as numbers and percentages, and were compared using the χ^2 test. A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI onset had uniform (no peak), unimodal (one peak) or bimodal distribution (two peaks), and the likelihood ratio test was then used to select the best circadian pattern among them.[11] In addition, the hierarchical likelihood ratio test was used to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier method was used to estimate survival curves of 1-year mortality according to AMI onset time (morning (6:00–11:59), afternoon (12:00–17:59), evening (18:00–23:59) and night-time (0:00–5:59)). The log-rank test was used to compare survival curves between the groups, and the Cox proportional hazards regression model was used to estimate HRs and 95% CIs. To reduce potential confounding effects due to patient background variability in the comparison between the afternoon-onset and other groups, a stratified Cox proportional hazards regression model was used, in which the potential confounding variables were included into the model as stratification factors. Cosinor analysis was used to estimate the amplitude of serum triglyceride (TG) levels on admission according to AMI onset time. Then, an F-test for the existence of a rhythm (amplitude) was used to examine whether the amplitude of serum TG levels on admission in patients with AMI had circadian variation or not. Statistical significance was set as $p<0.05$. All statistical analyses were performed using an in-house validated Fortran program or SAS V.9.3 (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

Bimodal circadian patterns of AMI onset in the overall population

The daily patterns of AMI onset in our cohort of 7755 patients were first analysed using the likelihood ratio test (figure 1). In the overall population, AMI onset clearly

Table 1 Demographics and clinical characteristics of the study population

	N=7755
Patients	
Age (years)	66 (57–74)
Male (%)	5872 (75.7)
Job (%)	3364 (48.2)
BMI (kg/m^2)	23.4 (21.4–25.7)
Cardiovascular risk factors	
Smoker (%)	4865 (63.9)
Drinker (%)	3321 (45.3)
Diabetes (%)	2586 (33.4)
Hypertension (%)	4424 (58.9)
Dyslipidaemia (%)	3259 (44.1)
Previous MI (%)	983 (13.0)
Angina pectoris (%)	1737 (23.4)
Multivessel disease (%)	2790 (38.4)
Collateral circulation (%)	2576 (35.7)
Clinical presentation	
Onset admission time <24 h (%)	6804 (89.1)
KILLIP ≥II (%)	1331 (18.0)
Initial TIMI ≤II (%)	4759 (68.4)
STEMI (%)	6567 (86.0)
Laboratory data on admission	
Blood glucose level (mg/dL)	152 (122–209)
HDL cholesterol (mg/dL)	44 (37–53)
LDL cholesterol (mg/dL)	121 (99–147)
Triglycerides (mg/dL)	92 (58–142)
HbA1c (%)	5.9 (5.5–6.9)
Peak CK (IU/L)	2147 (1069–4006)
eGFR (mL/min/1.73 m^2)	64.5 (49.2–80.9)
Localisation of MI	
LAD	3050 (41.7)
RCA	2447 (33.4)
LCX	998 (13.6)
LMT	164 (2.2)

Categorical variables are presented as number (%), and continuous variables are presented as quartile. Laboratory data were measured on admission. Smoker was defined as a patient with a smoking history, and drinker was defined as an active drinker. Number (%) of localisation of MI was calculated out of 7319 patients who underwent coronary angiography.
BMI, body mass index; CK, creatine kinase; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LAD, left anterior descending artery; LCX, left circumflex artery; LDL, low-density lipoprotein; LMT, left main trunk; MI, myocardial infarction; RCA, right coronary artery; STEMI, ST-elevation myocardial infarction.

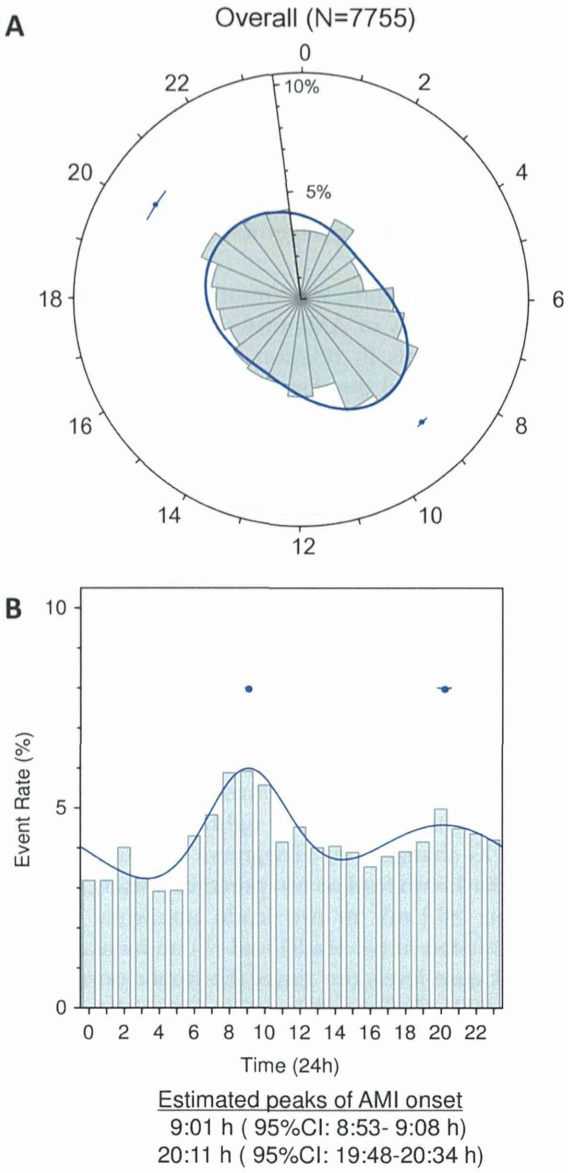

Figure 1 Circadian pattern of acute myocardial infarction (AMI) onset in the overall population. A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% CIs, respectively.

exhibited a circadian pattern consisting of two peaks (bimodal: p<0.001): a primary peak at 9:01 (95% CI 8:53 to 9:08) and a secondary peak at 20:11 (95% CI 19:48 to 20:34). The primary peak was more clearly defined than the secondary peak in the circular and columnar histograms (figure 1A, B, respectively).

The likelihood ratio test analysis revealed that the peak time of AMI onset varied according to the day of the week (figure 2). For example, the primary peak onset time was earliest on Monday (8:24 (95% CI 8:04 to 8:44)) and latest on Sunday (9:44 (95% CI 9:22 to 10:06)). On Tuesday, patients exhibited a circadian pattern of AMI onset characterised by late primary (9:28 (95% CI 9:06 to 9:51)) and secondary peak onset times (21:13 (95% CI 20:40 to 21:46)), whereas earlier peak onset times (8:43 (95% CI 8:15 to 9:10) and 19:09 (95% CI 18:23 to 19:55)) were detected on Thursday. Notably, the evening peak was higher and sharper than the morning peak on Saturday (figure 2).

Factors affecting the circadian patterns of AMI onset

The hierarchical likelihood ratio analysis revealed that serum TG levels on admission, smoking, age, drinking, blood glucose levels on admission, gender and working