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Original Article

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Circadian Rhythm of Subspecies of Low-Density Lipoprotein-Cholesterol and High-Density Lipoprotein-Cholesterol in Healthy Subjects and Patients with Type 2 Diabetes

Toshiyuki Hayashi[1, 2], Masumi Ai[3], Satoshi Goto[1], Marie Nakamura[3, 4], Hiroe Nagaike[1], Risa Suzuki[5], Yasuko Abe[3, 6], Motoko Ohta[7], Yasuki Ito[7] and Tsutomu Hirano[1, 8]

[1]Department of Medicine, Division of Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine. Tokyo, Japan
[2]Yurakubashi Clinic, Tokyo, Japan
[3]Department of Insured Medical Care Management, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan
[4]Koshigaya Laketown Clinic, Saitama, Japan
[5]Department of Family Medicine, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan
[6]Department of Internal Medicine, Yokufukai Hospital, Social Welfare Foundation Yokufukai, Tokyo, Japan
[7]Vaccine & Diagnostic R&D Department, Denka Co., Ltd., Niigata, Japan
[8]Diabetes Center, Ebina General Hospital Kawaharaguchi, Kanagawa, Japan

Aims: We established automated assay kits for quantifying small dense low-density lipoprotein (sdLDL)-cholesterol (C), LDL-triglyceride (TG), and high-density lipoprotein (HDL)3-C, and apolipoprotein (apo)E-rich HDL-C, and these have been recognized as sensitive biomarkers for predicting coronary artery disease. We investigated the circadian rhythms of these novel lipids to determine if fasting is required to determine basal levels.

Methods: Forty-eight inpatients with type 2 diabetes and 19 healthy volunteers were studied. Blood samples were collected at seven time points, which were obtained after an overnight fast, before and 2 h after each meal, and before the next breakfast. sdLDL-C, LDL-TG, remnant-like particle (RLP)-C, TG-rich lipoprotein (TRL-C), HDL3-C, and apoE-rich HDL-C were measured by the homogeneous methods. NonHDL-C, large buoyant (lb) LDL-C and HDL2-C were calculated by subtracting sdLDL-C from LDL-C or HDL3-C from HDL-C, respectively.

Results: Serum TG levels were significantly increased after meals in both healthy participants and patients with diabetes. RLP-C and TRL-C were also increased postprandially. LDL-TG, LDL-C, nonHDL-C, HDL2,3-C, and apoE-rich HDL-C did not exhibit significant fluctuation during the day in healthy participants and patients with diabetes. sdLDL-C was slightly increased postprandially in subjects with diabetes (1–2 mg/dl, 3%–9%), though its increase was not significant compared to the baseline (fasting) level. Significant postprandial reduction was observed with LDL-C and lbLDL-C. There was no influence of statin therapy or oral anti-diabetes drugs on the circadian rhythm of LDL-C subspecies.

Conclusions: Subtle postprandial increase in sdLDL-C is considered a negligible level in general clinical practice. Fasting is not mandatory to measure basal concentrations of LDL and HDL subspecies.

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Key words: LDL-cholesterol, HDL-cholesterol, Subspecies, Postprandial lipemia, Diabetes

Introduction

Diabetes is a leading cause for coronary artery disease (CAD), and the dyslipidemia developed in patients with diabetes is closely associated with the high prevalence of CAD independently of hyperglycemia[1]. The diabetic dyslipidemia is characterized by elevated triglyceride (TG), reduced high-density lipoprotein (HDL)-cholesterol (C), and preponderance of small dense low-density lipoprotein (sdLDL)[2]. In addition, patients with diabetes preferentially have TG-enriched lipoproteins across all

lipoprotein spectrums from TG-rich lipoproteins (TRLs) to HDL[3]. It is well known that sdLDL particles are more atherogenic than large buoyant (lb) LDL particles[4]. Our group established a full automated assay kit for the quantification of sdLDL-C levels[5], and this assay was employed by famous cohort studies, such as the Atherosclerosis Risk in Community (ARIC) study[6], Suita study[7], and Hisayama study[8]. All studies have consistently revealed that sdLDL-C is superior to LDL-C for predicting CAD. Moreover, we established a full automated LDL-TG assay kit[9]. Using our test kit, Saeed *et al.*[10] reported that LDL-TG is a sensitive marker for cardiovascular (CV) diseases in the ARIC study. Low HDL-C is a good predictor of CAD, but it is unclear whether HDL has a causal link to CAD[11]. The trend in HDL assessment shifts from HDL-C concentrations to the properties of HDL particles, especially to their ability to efflux cholesterol[12, 13]. Nevertheless, it is clinically difficult to measure the nature of HDL. HDL has subspecies, large cholesterol-enriched HDL2 and small cholesterol-depleted HDL3. It is generally accepted that small dense HDL, namely, HDL3, is more efficient for cholesterol efflux capacity and has more potent anti-atherogenic property than HDL2 [14]. In fact, Albers *et al.*[15] reported that HDL3-C but not HDL2-C is protective against CV events in the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) trial. HDL has another subspecies, apoE-rich or apoE-poor HDL[16]. ApoE-rich HDL is reported to have more potent power of cholesterol efflux from tissues than apoE-poor counterparts. ApoE-rich HDL has shorter residence time in blood than apoE-poor HDL, which correlates inversely with reverse cholesterol transport ability[17]. Thus, HDL3 and apoE-rich HDL have been paid attention for reflecting the anti-atherogenic nature of HDL particles.

LDL and HDL subspecies are expected to be widely used as sensitive biomarkers for the prediction of CAD. However, it is still uncertain whether these values are fluctuated in the day or keep constant regardless of meals. TRLs are naturally increased after meals, and its postprandial increase subsequently affects subspecies of LDL and HDL particles[18]. Therefore, it is important to examine the circadian rhythms of LDL and HDL subspecies and to know if fasting is needed to obtain their basal values. The aim of this study is to determine whether blood sampling time significantly affects subspecies of LDL-C and HDL-C. The recent trend in Europe and the United States is that fasting blood sampling is no longer mandatory[19]. Therefore, the results of this investigation may justify this policy.

Methods

Forty-eight inpatients (male:female, 29:19) with type 2 diabetes administered in Showa University Hospital and 19 healthy volunteers (male:female, 9:10) of medical staff in Tokyo Medical Dental University were studied. **Table 1** shows the clinical characteristics of patients with diabetes. There were no serious kidney, liver, endocrine, and infectious diseases in the patients. Twenty-eight patients with diabetes were taking statins. TG-lowering agents, ezetimibe, or resin had not been prescribed. Insulin users and glucagon-like peptide-1 receptor agonists users were not included. Standard medication for the patients was not discontinued on the examination day. Patients were given a calorie-restricted diet (27–28 kcal/ideal body weight kg) specified by a dietitian. Healthy volunteers were recruited at Tokyo Medical Dental University. The health status of healthy participants was evaluated by a recent medical check-up. They were basically healthy, and diabetes was not included. There was no restriction of food intake for healthy subjects.

After overnight fasting, blood samples were collected at seven time points, which were obtained before and 2 h after each meal and before breakfast in the following day. All blood samples for controls and patients with diabetes were taken in a sitting position and collected at 8:00, 10:00, 12:00, 14:00, 18:00, 20:00, and next 8:00, and serum samples were stored at −80℃ until the day of the assay. The area under the curve (AUC) during the day was calculated by the trapezium method. The incremental area under the curve (iAUC) was calculated by subtracting fasting AUC from total AUC. sdLDL-C[5], LDL-TG[9], HDL3-C[20], apoE-rich HDL-C[21], and remnant-like particle (RLP)-C[22] were measured by the homogeneous method (Denka Co., Ltd. Tokyo, Japan). TRL-C was measured directly by the homogeneous method (Denka Co., Ltd.) using a two-step process where LDL and HDL are degraded, and

Address for correspondence: Tsutomu Hirano, Diabetes Center, Ebina general hospital, Kawaharaguchi Ebina City, Kanagawa 243-0433, Japan.
E-mail: hirano@med.showa-u.ac.jp

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Table 1. Clinical characteristics of patients with type 2 diabetes

n (Male/Female)	48 (29/19)
Age (years)	66 ± 11
Duration of diabetes (years)	9.5 ± 8.5
Body mass index (kg/m^2)	24.9 ± 4.2
AST (IU/L)	27 ± 16
ALT (IU/L)	33 ± 29
γ-GTP (IU/L)	39 ± 30
eGFR (mL/min/1.73m^2)	76 ± 17
Fasting Glucose (mg/dl)	154 ± 37
Fasting IRI (μU/ml)	6.2 ± 4.6
HbA1c (%)	8.6 ± 1.2
Neuropathy/Retinopathy/Nephropathy, *n* (%)	25/12/6 (52/25/12)
Coronary artery disease/Stroke, *n* (%)	6/5 (12/10)
Medications *n* (%)	
Sulfonylureas	12 (25)
Glinide	2 (4)
DPP4 inhibitors	32 (66)
Metformin	24 (50)
Pioglitazone	3 (6)
SGLT2 inhibitors	7 (14)
Statins	28 (58)

Data are expressed as mean (mg/dL) ± standard deviation
IRI = immunoreactive insulin

the cholesterol content of the remaining lipoproteins is measured[23]. Total-C, TG, LDL-C, and HDL-C were measured using a commercially available assay kit (Denka Co., Ltd.). lbLDL-C was estimated by subtracting sdLDL-C from LDL-C. HDL2-C was calculated by subtracting HDL3-C from HDL-C. NonHDL-C was obtained by subtracting HDL-C from Total-C. Fasting immunoreactive insulin was measured by the ELISA method.

The study was approved by the institutional review board of Showa University School of Medicine and Tokyo Medical and Dental University School of Medicine. Written informed consent was obtained from all participants including healthy volunteers. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Statistical Analysis

Variables are expressed as mean ± standard deviation, standard error (SE), or proportion. Comparison between fasting value and nonfasting value was determined by paired *t*-test or Wilcoxon signed-rank test. Correlation between two variables was determined by the Pearson's correlation coefficient. A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were performed using JMP software version 15 (SAS Institute, Cary, NC, USA).

Results

Fig. 1 and Fig. 2 depict the absolute and percent changes in concentrations of TG, RLP-C, TRL-C, LDL-TG, LDL-C, sdLDL-C, lbLDL-C, nonHDL-C, HDL-C, HDL2-C, HDL3-C, and apoE-rich HDL-C values during the day in healthy participants. Serum TG level increased after meals and peaked 2 h after supper. The maximum change from the fasting value was 65% (mean: 93–150 mg/dl). RLP-C level elevated after breakfast and peaked 2 h after lunch. The maximum change was 63% (3.1–5.1 mg/dl). TRL-C level increased slightly after meals and peaked 2 h after supper. The maximum change was 23% (21.9–25.5 mg/dl). LDL-C and lbLDL-C levels decreased slightly after breakfast, and the reductions were approximately 6 mg/dl (6%). sdLDL-C and LDL-TG levels decreased slightly after breakfast, but these reductions were only 1 mg/dl (5%). Concentrations of nonHDL-C, HDL-C, HDL2-C, HDL3-C, and apoE-rich HDL-C did not change during the day. **Supplemental Fig. 1** shows the absolute bias plot from the baseline. Significant changes from the baseline were similar to those in **Fig. 1**.

Fig. 3 and Fig. 4 depict the absolute and percent changes in concentrations of TG, RLP-C, TRL-C,

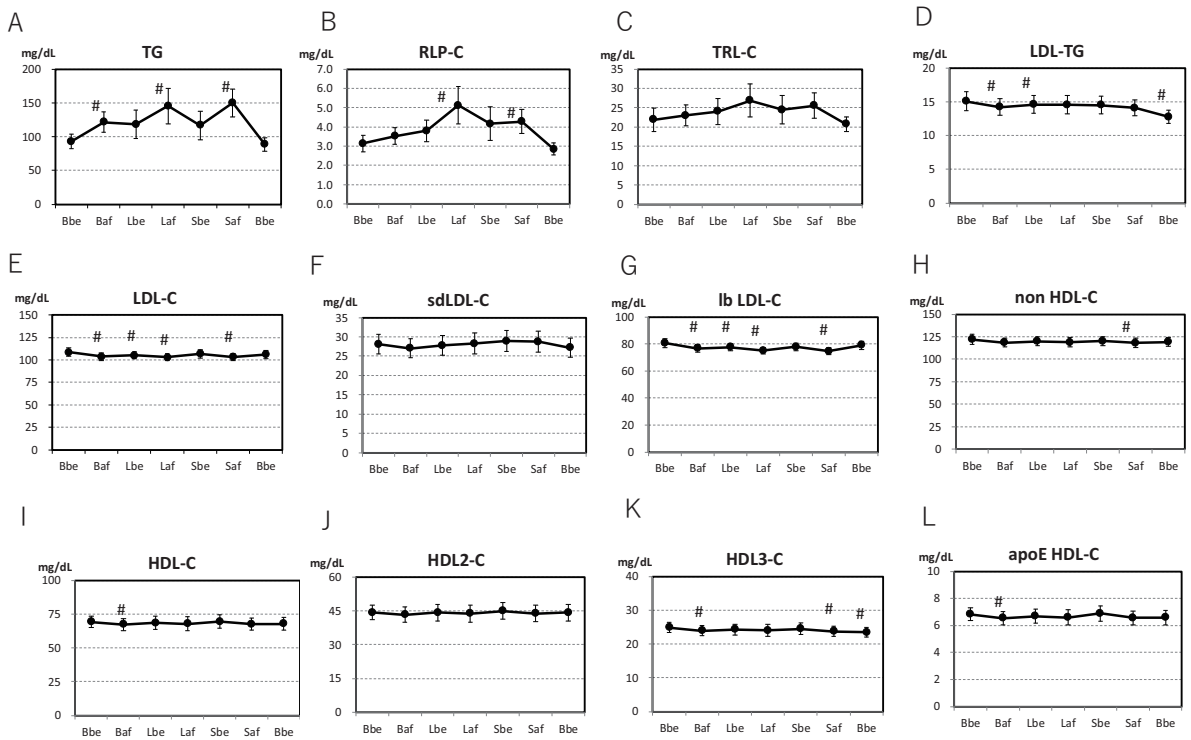

Fig. 1. Change in concentration of serum lipids during the day in healthy participants

A, Triglycerides (TG). B, Remnant-like particle cholesterol (RLP-C). C, Triglyceride-rich lipoprotein-cholesterol (TRL-C). D, Low-density lipoprotein-triglyceride (LDL-TG). E, Low-density lipoprotein-cholesterol (LDL-C). F, Small dense low-density lipoprotein-cholesterol (sdLDL-C). G, Large buoyant low-density lipoprotein-cholesterol (lbLDL-C). H, Non-high-density lipoprotein-cholesterol (nonHDL-C). I, High-density lipoprotein-cholesterol (HDL-C). J, High-density lipoprotein2-cholesterol (HDL2-C). K, High-density lipoprotein3-cholesterol (HDL3-C). L, Apolipoprotein-rich high-density lipoprotein-cholesterol (apoE HDL-C).
The individual time points correspond to Bbe (before breakfast), Baf (after breakfast), Lbe (before lunch), Laf (after lunch), Sbe (before supper), and Saf (after supper). Each value is presented as mean ± SE. #: $p < 0.05$ compared with before breakfast on the first day.

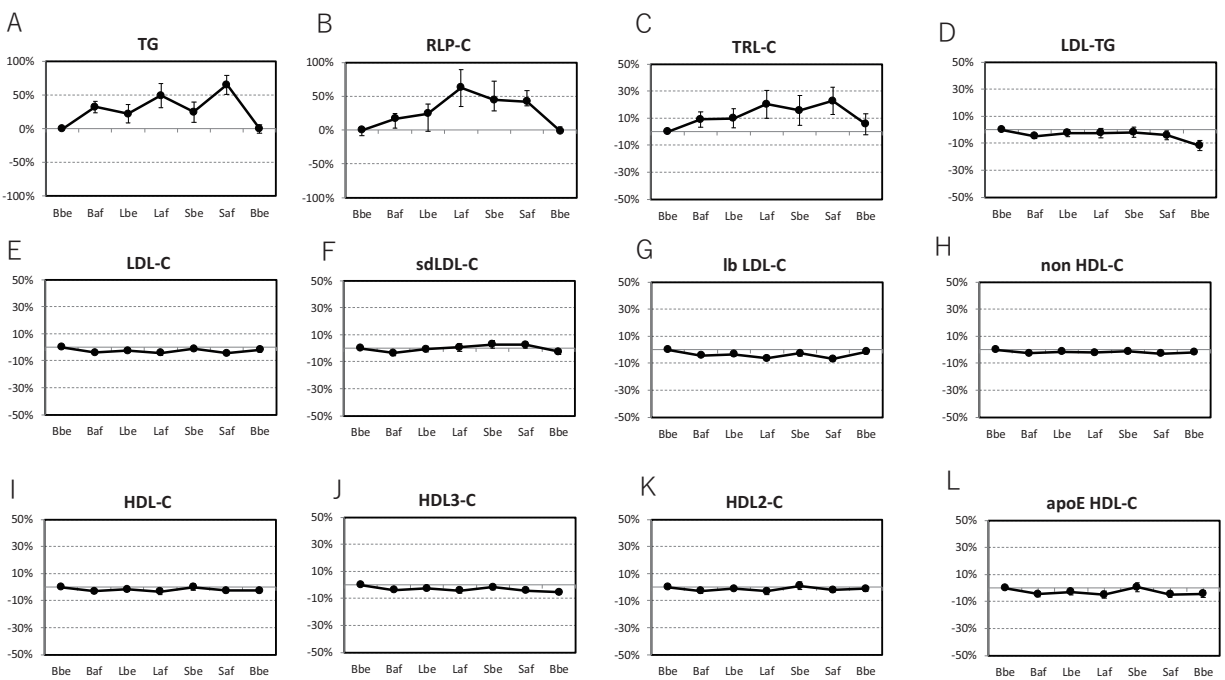

Fig. 2. Percent change in serum lipids during the day in healthy participants

Each value is presented as mean ± SE.

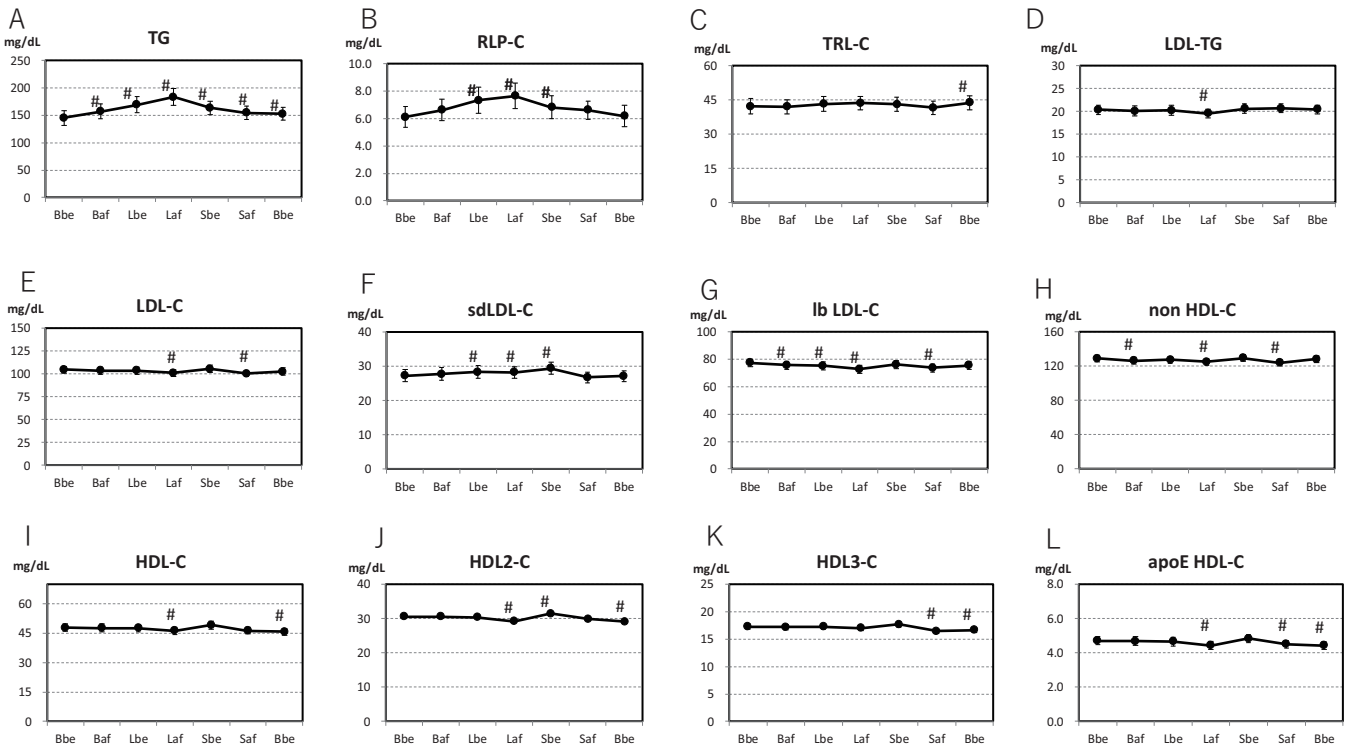

Fig. 3. Change in concentration of serum lipids during the day in patients with type 2 diabetes

Each value is presented as mean ± SE. #: $p < 0.05$ compared with before breakfast on the first day.

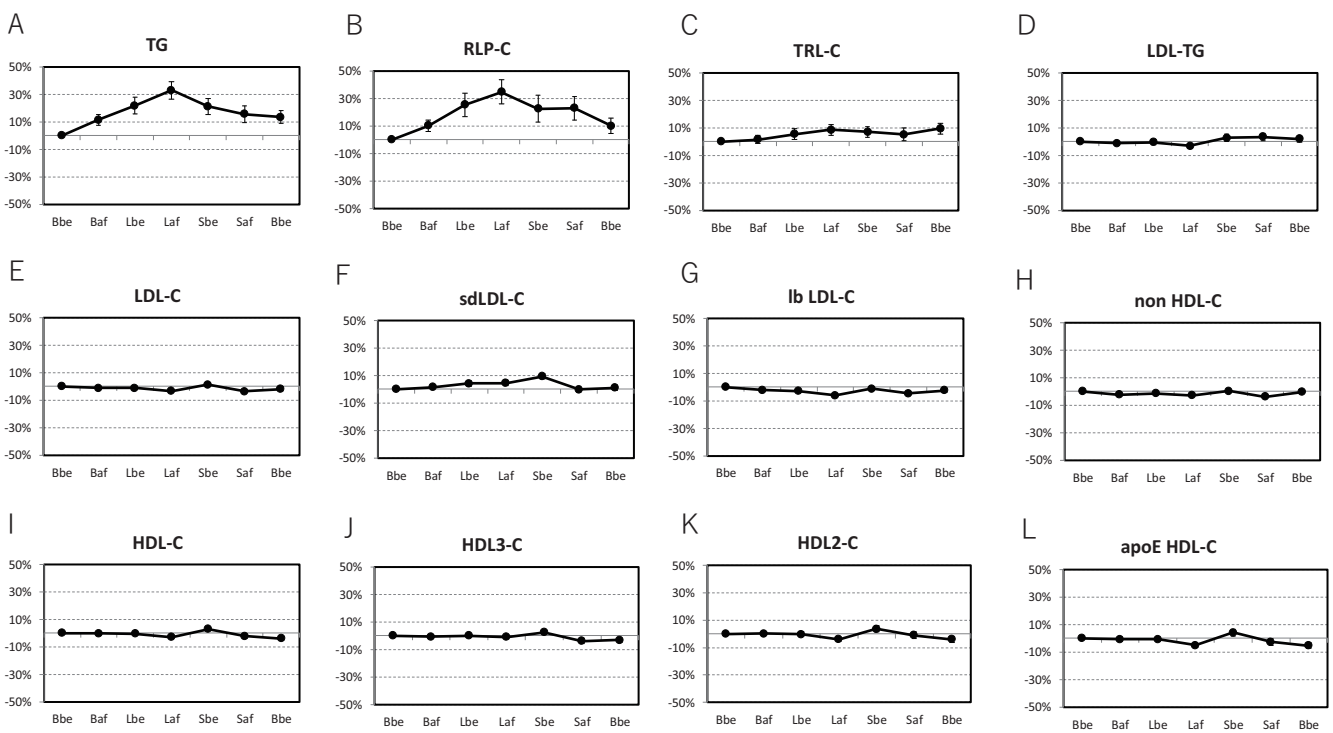

Fig. 4. Percent change in serum lipids during the day in patients with type 2 diabetes

Each value is presented as mean ± SE.

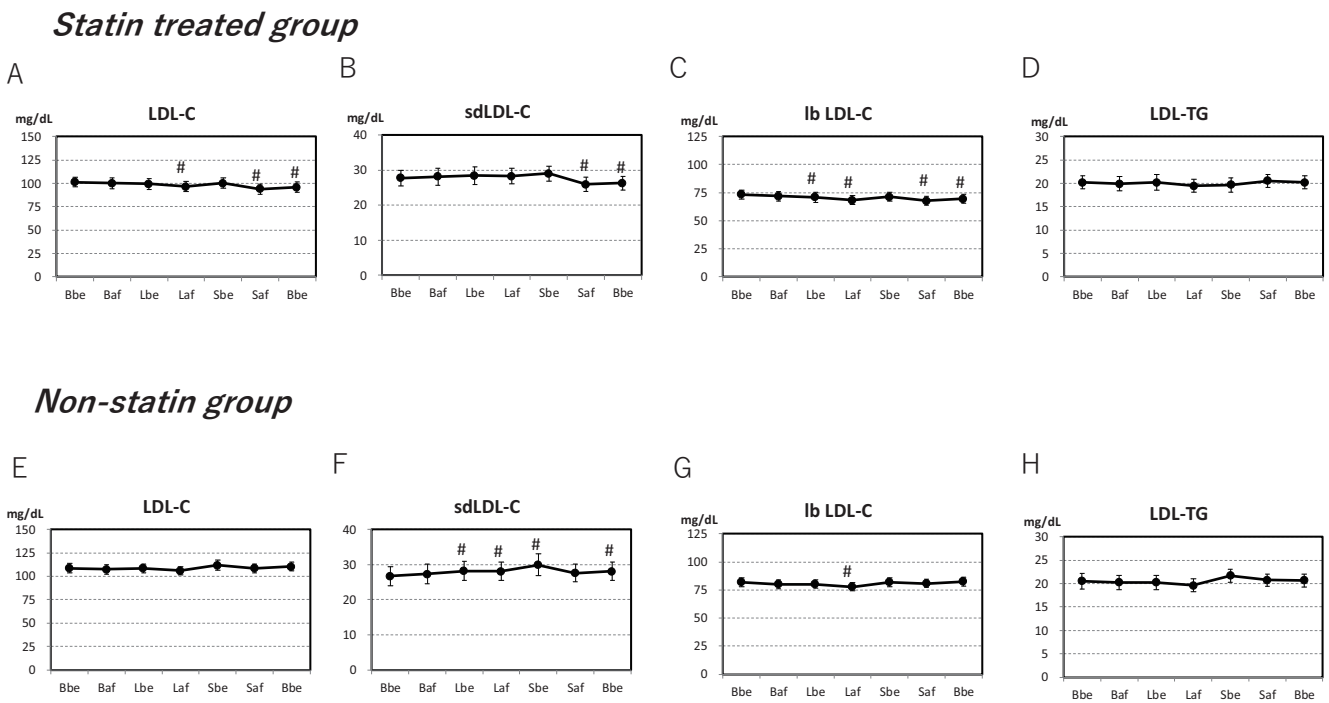

Fig. 5. Change in LDL subspecies lipid levels during the day in patients with type 2 diabetes stratified by statin treatment

A to D, Statin treatment group. E to H, Non-statin group. A and E, LDL-TG. B and F, LDL-C. C and G, lbLDL-C. D and H, sdLDL-C. Each value is presented as mean ± SE. #: $p < 0.05$ compared with before breakfast on the first day.

LDL-TG, LDL-C, sdLDL-C, lbLDL-C, nonHDL-C, HDL-C, HDL2-C, HDL3-C, and apoE-rich HDL-C during the day in patients with type 2 diabetes. Serum TG levels gradually increased after breakfast and peaked 2 h after lunch. The maximum change was 33% (145–183 mg/dl). RLP-C level elevated after breakfast and peaked 2 h after lunch. The maximum change was 35% (6.1–7.7 mg/dl). TRL-C and LDL-T levels did not change significantly after meals. sdLDL-C level increased slightly after breakfast and peaked 2 h after supper. However, the increase was only 2 mg/dl (9%). LDL-C and lbLDL-C levels decreased slightly after meals, and these reductions were approximately 5 mg/dl (5%). Concentrations of nonHDL-C, HDL-C, HDL2-C, HDL3-C, and apoE-rich HDL-C did not change during the day. **Supplemental Fig. 2** shows the absolute bias plot from the baseline. Significant changes from the baseline were similar to those in **Fig. 3**.

Fig. 5 shows the changes in LDL-TG, LDL-C, sdLDL-C, and lbLDL-C concentrations in patients with type 2 diabetes stratified by statin use. In the statin-treated group, LDL-C and lbLDL-C levels decreased slightly after meals, and these reductions were approximately 6 mg/dl (7%). sdLDL-C decreased after dinner, but these reductions were only 2 mg/dl (6%). LDL-TG did not change during the day. The same tendency was seen in the non-statin group, but the postprandial changes in LDL-C and lbLDL-C levels were even smaller. sdLDL-C increased slightly after meals, and this increment was approximately 3 mg/dl (11%). The postprandial increase of sdLDL-C was not significantly different between statin users and non-statin users (data not shown). The use of insulin secretagogues (sulfonylureas, glinides, and dipeptidyl peptidase-4 (DPP4) inhibitors) or the use of insulin sensitizers (metformin and pioglitazone) may affect the circadian rhythm of LDL subspecies. However, as shown in **Supplemental Fig. 3**, there was no significant change in the circadian rhythm of the LDL subspecies in the subset treated with insulin secretagogues, insulin sensitizers, or both. Sodium-glucose cotransporter-2 (SGLT-2) inhibitors can alter circulating serum volume and might cause fluctuation in serum lipoproteins. As shown in **Supplemental Fig. 3**, the use of SGLT-2 inhibitors did not affect the circadian rhythm of the LDL subspecies.

Table 2 shows a comparison of mean serum lipid concentrations between two fasting and five nonfasting samples and iAUC of serum lipids during the day. In healthy participants, nonfasting values of TG and RLP-C were significantly higher than fasting values, whereas nonfasting values of LDL-C and

Table 2. Comparison between fasting value and average values of non-fasting in serum lipids and the incremental area under the curve (iAUC) in a day

Healthy participants					
	Fasting (mean of 2point)	Non-fasting (mean of 5point)	*p*-value	iAUC	*p*-value
TG	68 [62 , 120]	93 [67 , 199]	0.0002	383 [224 , 1155]	0.0001
Total-C	190 ± 23	187 ± 21	0.1649	-50 ± 133	0.1177
LDL-C	108 ± 20	105 ± 18	0.0185	-61 ± 101	0.0166
sdLDL-C	24.9 [19.3 , 33.2]	25.5 [19.3 , 35.4]	0.7086	11.8 [-20.1 , 32.2]	0.4474
lb LDL-C	80.4 [70.2 , 91.3]	76.3 [67.6 , 85.3]	0.0039	-48.5 [-93.9 , -30.7]	0.0024
LDL-TG	11.8 [9.8 , 17.3]	12.6 [10.0 , 16.3]	0.4171	0.2 [-13.1 , 10.1]	0.7983
RLP-C	2.4 [2.1 , 3.2]	3.0 [2.3 , 5.7]	0.0056	15.3 [-1.3 , 23.3]	0.0038
TRL-C	17.1 [14.5 , 27.3]	18.5 [13.8 , 33.5]	0.0874	49.0 [-21.7 , 144.0]	0.0728
HDL-C	68.5 ± 19.2	68.1 ± 20.7	0.6193	-11.2 ± 60.1	0.4273
HDL2-C	44.3 ± 14.7	44.0 ± 15.5	0.5620	-4.8 ± 40.7	0.6108
HDL3-C	24.2 ± 6.4	24.1 ± 7.0	0.7288	-6.4 ± 22.5	0.2327
apoE HDL-C	6.7 ± 2.2	6.6 ± 2.3	0.3900	-1.8 ± 7.5	0.3082
non HDL-C	121 ± 23	119 ± 22	0.1535	-39 ± 91	0.0778
Diabetic patients					
	Fasting (mean of 2point)	Non-fasting (mean of 5point)	*p*-value	iAUC	*p*-value
TG	127 [101 , 1646]	151 [113 , 184]	< 0.0001	239 [71 , 428]	< 0.0001
Total-C	176 ± 30	174 ± 30	0.0790	-52 ± 138	0.0122
LDL-C	104 ± 25	103 ± 25	0.2182	-29 ± 105	0.0617
sdLDL-C	24.9 [19.6 , 31.3]	25.6 [21.3 , 31.9]	0.0285	8.5 [-17.5 , 32.3]	0.1622
Ib LDL-C	79.5 [62.1 , 89.0]	79.0 [58.1 , 89.0]	0.0008	-38.1 [-97.7 , 3.5]	0.0004
LDL-TG	19.5 [15.4 , 24.6]	19.2 [15.3 , 23.8]	0.3722	-1.2 [-17.7 , 12.3]	0.5920
RLP-C	4.6 [3.6 , 6.5]	5.3 [4.1 , 7.6]	0.0013	12.2 [1.5 , 23.0]	0.0002
TRL-C	37.0 [29.8 , 52.8]	37.5 [30.4 , 49.6]	0.6356	-3.1 [-58.0 , 52.8]	0.6935
HDL-C	46.8 ± 12.6	47.4 ± 12.7	0.1279	0.6 ± 50.6	0.9380
HDL2-C	29.9 ± 10.6	30.3 ± 10.6	0.1069	2.5 ± 33.8	0.6104
HDL3-C	17.0 ± 3.0	17.1 ± 3.0	0.3960	-1.9 ± 20.6	0.5205
apoE HDL-C	4.6 ± 1.6	4.6 ± 1.6	0.6428	-0.4 ± 7.4	0.7318
non HDL-C	129 ± 27	126 ± 27	0.0059	-53 ± 98	0.0005
Glucose	154 ± 36	200 ± 48	< 0.0001	887 ± 521	< 0.0001

Data are expressed as mean (mg/dl) ± standard deviation or median (mg/dl) [25% and 75% quartiles]. iAUC = incremental area under the curve. See the legend in Fig. 1 for abbreviations.

lbLDL-C were lower than fasting values. Concentrations of Total-C, sdLDL-C, LDL-TG, TRL-C, HDL-C, HDL2,3-C, apoE-rich HDL-C, and nonHDL-C were not significantly different between fasting and nonfasting. In subjects with diabetes, nonfasting values of TG and RLP-C were significantly higher than fasting values, whereas nonfasting values of lbLDL-C and nonHDL-C were lower than fasting values. sdLDL-C level was slightly higher in nonfasting than in fasting, but this difference was only 0.7 mg/dl (3%). Concentrations of Total-C, LDL-C, LDL-TG, TRL-C, HDL-C HDL2,3-C, and apoE-rich HDL-C were not significantly different between fasting and nonfasting. iAUC indicates postprandial accumulation or consumption of lipid during the day above the baseline (fasting). In healthy participants, significant postprandial accumulation was observed in TG and RLP-C, whereas postprandial loss was observed in LDL-C and lbLDL-C. There was no significant variation of Total-C, TRL-C, LDL-TG, sdLDL-C, nonHDL-C, and HDL-C subspecies during the day. In participants with diabetes, significant postprandial accumulation was observed in TG and RLP-C, whereas postprandial loss was observed in Total-C, lbLDL-C, and nonHDL-C. There was no significant accumulation or loss of LDL-

C, LDL-TG, sdLDL-C, and HDL-C subspecies during the day. iAUC of TG was significantly correlated with iAUC of lbLDL-C (r=−0.584, $p<0.0086$), but insignificantly correlated with iAUC of sdLDL-C (r=0.407, $p=0.081$) in healthy subjects. iAUC of LDL-C was highly correlated with iAUC of lbLDL-C in healthy subjects and patients with diabetes (r=0.871 and r=0.968, respectively, $p<0.0001$). **Table 2** shows the fasting, nonfasting, and iAUC of glucose levels for patients with diabetes. These glucose parameters were not correlated with iAUC of the LDL and HDL subspecies (data not shown).

Discussion

Lipoprotein kinetic studies using stable isotope have revealed that the residence time of sdLDL is much longer than that of lbLDL. Campos *et al.*[24] reported a plasma residence time of apoB-100 within dense LDLs (d=1.036–1.063 g/ml) of 2.44 days and in light LDLs (d=1.019–1.036 g/ml) of 1.69 days in women with no diabetes. Thongtang *et al.*[25] reported a plasma residence time of apoB-100 within dense LDLs (d=1.044–1.063 g/ml) of 3.10 days and in light LDLs (d=1.019–1.044 g/ml) of 1.95 days in subjects with combined hyperlipidemia. Long residence time of sdLDL over 2 days suggests that serum concentrations are not easily changed during the day. Participants with diabetes exhibited slight fluctuation of sdLDL-C, but the maximum change was only 9%. It has been generally accepted that fasting is not required for direct LDL-C measurement. Nordestgaard *et al.* reported that LDL-C is decreased by a maximum of 8 mg/dl after habitual food intake based on the data from large number of individuals participating in the Copenhagen City Heart study and concluded that minor and transient changes in LDL-C concentrations are clinically insignificant[26]. They speculated that a drop in LDL-C is due to fluid intake that dilutes serum concentration. We found that lbLDL-C and LDL-C levels tended to decrease after meals, whereas sdLDL-C tended to be increased. Therefore, the postprandial reduction in LDL-C is not simply due to serum dilution. iAUC analysis suggests that a decrease in lbLDL-C is associated with a postprandial increase in serum TG. Brunzell *et al.*[27] hypothesized that endogenous and exogenous TG compete against each other for a saturable TG removal system. According to this hypothesis, a postprandial increase in chylomicrons interferes with the VLDL–LDL lipolytic cascade, thereby reducing LDL production from VLDL. Since lbLDL is produced from VLDL before sdLDL is produced[4], lbLDL may be susceptible to postprandial increases in TG. Postprandial reduction of LDL-C has been observed in many literatures. iAUC analysis revealed that the decrease in LDL-C was due to the decrease in lbLDL-C. Because postprandial changes in sdLDL-C were equal to or less than LDL-C, we concluded that subtle changes in sdLDL-C were negligible in general clinical practice. Previously, our group reported slight circadian rhythm of sdLDL-C in 20 healthy subjects, where plasma concentration of sdLDL-C was the highest at fasting and decreased after meals[28]. Similar results were reported by Hirayama and Miida[29] in 10 healthy subjects and 15 subjects with type 2 diabetes. However, in these studies, sdLDL-C was measured using the precipitation method[30]. The precipitation method consists of two steps: (1) to precipitate apoB-containing particles excepting sdLDL with heparin-magnesium and (2) to measure LDL-C in the supernatant. There is a possibility that postprandial increase in TG affects the precipitation process to discriminate sdLDL from other apoB-containing lipoproteins. In this study, sdLDL-C concentration was determined by the homogeneous method, which is different in assay principle from the precipitation method. We confirmed that the sdLDL-C values measured in the homogenous assay were stable with TG up to 1000 mg/dl[5]. The difference in measurement method may have influenced the difference in circadian rhythm.

LDL-TG levels have been reported to be significantly correlated with serum TG levels[9]. Therefore, a postprandial increase in serum TG may increase LDL-TG and cause its fluctuations. Recently, we investigated the characteristics of LDL-TG in many patients with type 2 diabetes and found that LDL-TG was strongly correlated with apoB but weakly correlated with TG[31]. Here we found that there was no significant change in LDL-TG levels during the day. This suggests that lipid exchange between LDL and TRL takes longer than expected. However, few participants in the current study had remarkable postprandial hyperlipidemia, so it cannot be ruled out a possibility that severe chylomicronemia rapidly increases LDL-TG.

HDL particles are composed of apoA1 and apoAII and have a very long residence time in blood circulation (3.8–5.5 days)[32]. However, HDL-C can change during the day due to lipid transfer between HDL and other lipoproteins. In particular, apoE-rich HDL has a short blood residence time[17] and can be variable. The study found that HDL2,3-C and apoE-rich HDL-C did not fluctuate during the day. Therefore, fasting is not required to measure basal levels of HDL-C subspecies.

More than half of the subjects with diabetes are treated with statins. Statins significantly reduce LDL residence time, regardless of lbLDL and sdLDL[23]. Reducing residence time can create a circadian rhythm for LDL variants. It was found that statin treatment does not affect changes in LDL-C subspecies. Statins are highly recommended for patients with diabetes and CAD. Current results suggest that sdLDL-C or LDL-TG determined by the homogeneous method is a reliable biomarker with no significant fluctuation, regardless of statin treatment.

Our current research has some limitations. First, all subjects with diabetes were receiving oral anti-diabetics that could affect serum lipid fluctuations. Second, all participants with diabetes were inpatients, and their diets were managed by a dietitian according to the guidelines of the Japan Diabetes Association. This may explain why postprandial increases in TG, RLP-C, and TRL-C were lower in subjects with diabetes than in healthy subjects. Therefore, current data for subjects with diabetes are limited in application to outpatients. Third, there was a lack of basic laboratory data for healthy participants. Nevertheless, these limitations do not undermine the credibility of our main consequences.

Conclusions

sdLDL-C, LDL-TG, and apoE-rich HDL did not show a significant circadian rhythm. Fasting is not mandatory to determine the basal concentrations of LDL-TG and subspecies of LDL-C and HDL-C.

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Other authors do not have COI.

Authors' Contributions

TH conducted this study, analyzed the data, and wrote. MA conducted this study, analyzed the data. SG, MN, HN, RS and YA conducted this study and collected data. NS and YI analyzed the data. TH wrote and finalized the paper. All authors critically revised the manuscript for important intellectual content and approved the final version of the manuscript.

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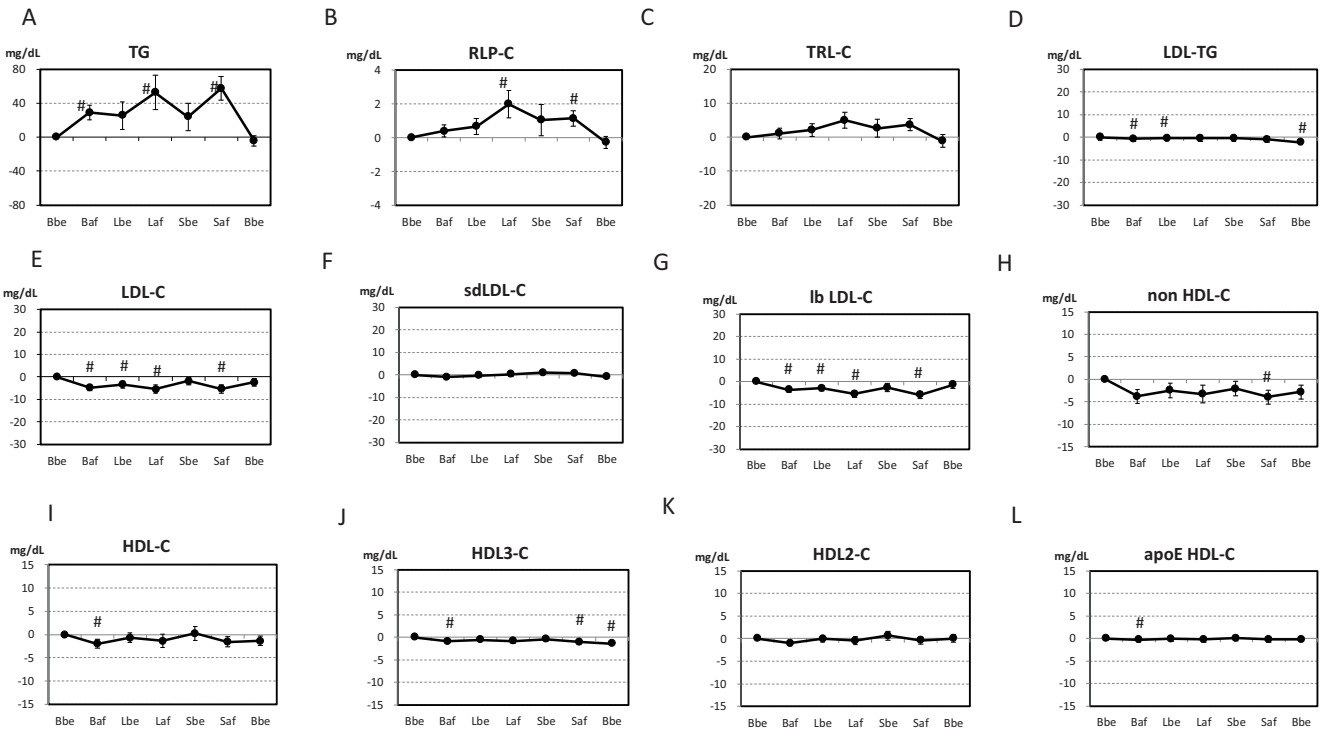
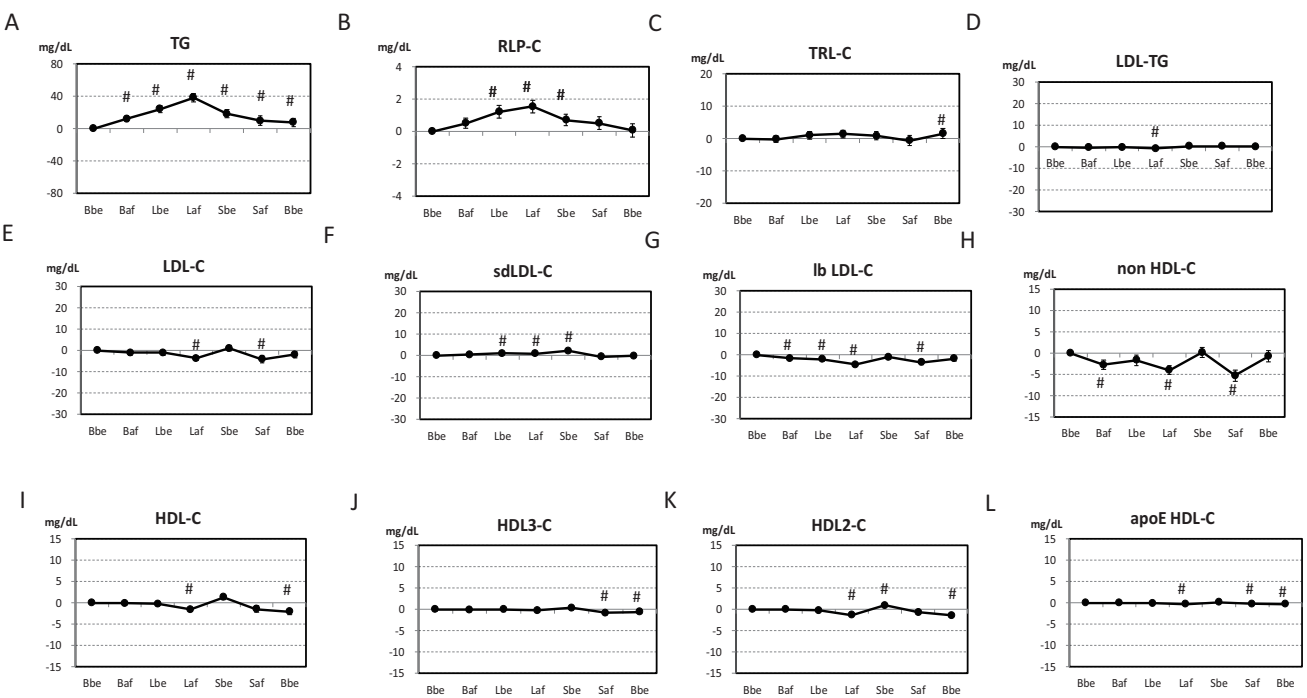
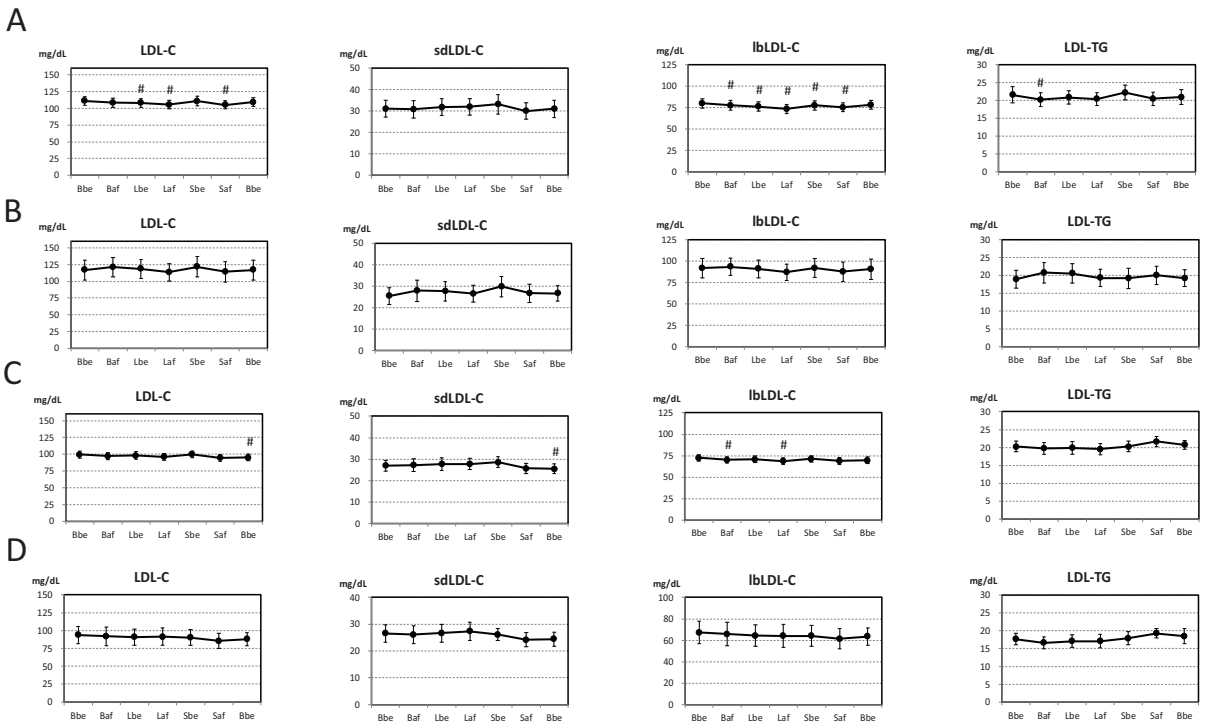


Supplemental Fig. 1. The absolute bias plot from the baseline in healthy participants



Supplemental Fig. 2. The absolute bias plot from the baseline in patients with type 2 diabetes



Supplemental Fig. 3. Circadian rhythm of the LDL subspecies in the subset treated with different oral anti-diabetes drugs

A: Insulin secretagogues (sulfonylureas, glinides, and dipeptidyl peptidase-4 (DPP-4) inhibitors) $n=15$
B: Insulin sensitizers (metformin and pioglitazone) $n=5$
C: Insulin secretagogues $n=20$
D: Sodium-glucose cotransporter-2 (SGLT-2) inhibitors $n=7$