Potential Impact on Lipoprotein Subfractions in Type 2 Diabetes

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ABSTRACT

INTRODUCTION: Recently, the sodium-glucose cotransporter2 (SGLT2) inhibitor empagliflozin has been shown to lower cardiovascular risk among diabetic patients. It is intriguing that some SGLT2 inhibitors have been found to increase low-density lipoprotein (LDL) cholesterol levels, while the relevance to high-density lipoprotein (HDL) cholesterol is unknown. Although the inhibitory effect of SGLT2 inhibitors on glucose reabsorption may accelerate compensatory lipid metabolism and subsequently reduce body weight and affect the lipid profile, much remains unclear about this mechanism. Therefore, we conducted this study to investigate in detail how canagliflozin affects lipoprotein fractions including LDL and HDL subclasses.

MATERIALS AND METHODS: This study is a multicenter prospective study. The participants were patients with 22 type 2 diabetes (60.7 ± 11.6 years, 59.1% of men) who had HbA1c ⩾ 7.0% and consented to participate in the study. They were administered 100 mg canagliflozin orally once per day. Biochemistry test and cholesterol levels of 20 lipoprotein fractions (G1-G20) using high performance liquid chromatography methods were examined before and after 12 weeks of treatment period.

RESULTS: Significant decreases were observed in the participants' body weight (69.7 to 67.9 kg, $P < .001$), systolic blood pressure (129.3 to 119.5 mm Hg, $P < .01$), and HbA1c (8.5% to 7.4%, $P < .001$). Cholesterol levels in the 20 lipoprotein fractions increased for very large HDL (G14, G15) and large HDL (G16) ($P < .05$).

CONCLUSIONS: Reduction in body weight, improvement of blood glucose levels, and increases in very large HDL and large HDL subclasses were observed after canagliflozin treatment. These beneficial changes might contribute to subsequent suppression of cardiovascular outcomes.

KEYWORDS: Canagliflozin, SGLT2 inhibitor, lipid metabolism, 20 lipoprotein fractions, large HDL, LDL subclasses

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Introduction

The sodium-glucose cotransporter2 (SGLT2) inhibitors are pharmacologic inhibitors that inhibit the SGLT2 function. In a large-scale clinical study, EMPA-REG OUTCOME, evaluating the safety of 1 of SGLT2 inhibitors, empagliflozin, in the cardiovascular system has been shown to have a significant reduction in risks for primary endpoints including cardiovascular mortality compared with the control groups.[1]

The sodium-glucose cotransporter2 inhibitor, canagliflozin, was found to increase low-density lipoprotein-cholesterol (LDL-C) levels during its development.[2] Similar observations have been reported with other inhibitors of SGLT2, suggesting the possibility of a class effect.[3] The mechanism of action is thought to involve the inhibition of glucose reabsorption exerted by SGLT2 inhibitors, leading to an enhancement of compensatory lipid metabolism, thereby affecting body weight reduction and lipid profiles as secondary effects. It is also reported that SGLT2 inhibitor reduces glucose metabolism while enhancing use of lipids, ketones, and branched-chain aminoacids.[4] However, few detailed investigations on such changes in lipid profiles caused by SGLT2 inhibitor have been performed.[2,3] In addition, although coronary artery protective effect of high-density lipoprotein–cholesterol (HDL-C),[5] especially large HDL-C has been reported,[6–8] there are few reports on the effect of SGLT2 inhibitor on HDL-C.

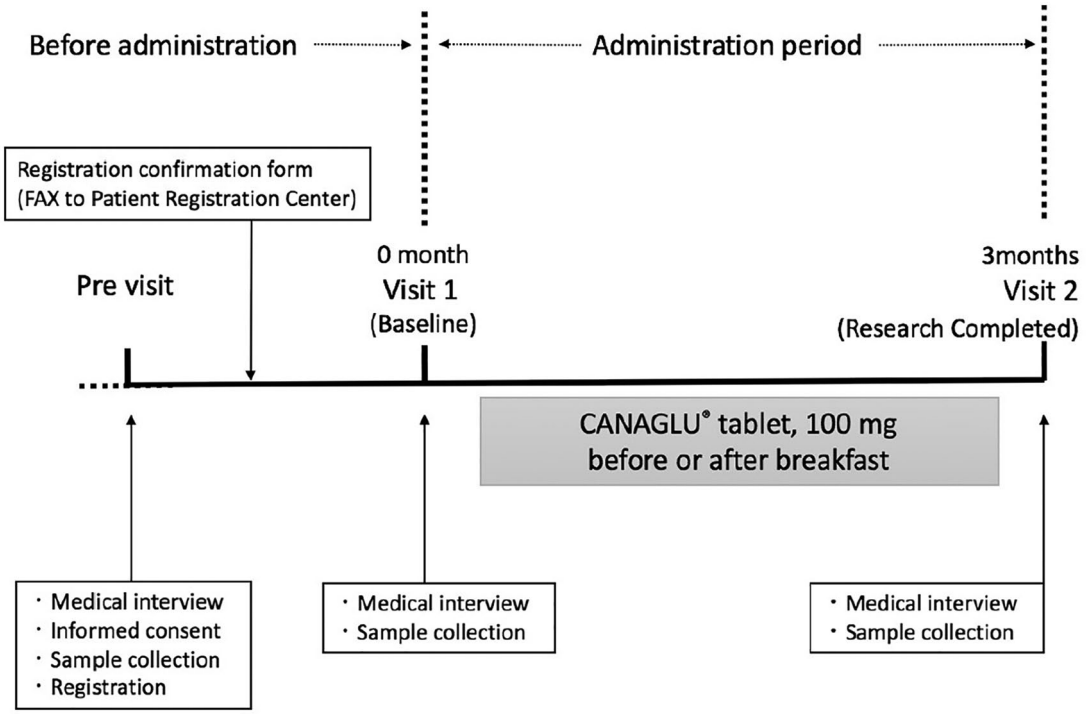

Figure 1. The study design: Administration of canagliflozin (CANAGLU Tablets 100 mg) to patients was carried out starting the day after visit 1. Blood tests took place at visit 1 (baseline) and visit 2 (end of the study after 3 months of drug administration).

In this study, we investigated changes in lipids as well as 20 lipoprotein fractions after 3 months of repeated administrations of SGLT2 inhibitor, canagliflozin, in patients with type 2 diabetes.

Aims

In this study, we aimed to examine the details of the effects of SGLT2 inhibitors on lipid profiles. For this, we orally administered 100 mg of canagliflozin per day in patients with type 2 diabetes for 3 months and examined changes in lipid data (triglyceride [TG], total cholesterol [TC], apoprotein-B [Apo-B]), cholesterol in 4 main lipoprotein fractions (chylomicron [CM], very-low-density lipoprotein [VLDL], LDL, HDL), cholesterol in 20 detailed lipoprotein fractions (G1-G20) before and after the drug administration. Cholesterols and neutral lipids contained in lipoprotein, main fractions (CM, VLDL, LDL, HDL), and 20 detailed lipoprotein fractions (subclass determined by the particle size) were analyzed using gel filtration high performance liquid chromatography (HPLC) methods.[9]

Materials and Methods

Participants

This study is a multicenter study conducted at 4 clinical facilities and 2 research facilities. The participants were recruited at 4 clinical facilities (Medical Hospital of Tokyo Medical and Dental University, Tokyo, Japan; Asano clinic, Saitama, Japan; Fukuoka University Chikushi Hospital, Fukuoka, Japan; Sugi Hospital, Fukuoka, Japan.). The surveyed population satisfied the following inclusion criteria: (1) patients who were suffering from type 2 diabetes, (2) patients between the age of 20 and 75, and (3) patients who had an HbA1c value (NGSP) of ⩾7.0%. Patients characterized by (1) any acute disorders or infectious diseases; (2) hospitalization; (3) prior to or post-surgery; (4) contraindicated for canagliflozin (Canaglu tablet, 100 mg), that is, with a history of hypersensitivity to the ingredients of the investigational drug, severe ketosis, diabetic coma or pre-coma, taking diuretic drugs, with renal dysfunction, estimated glomerular filtration rate (eGFR) below 45 mL/min/1.73 m2 or on dialysis[10]; or (5) taking fibrates, pioglitazone (Actos), or SGLT2 inhibitors were excluded from the study. The study was approved by the Ethics Committee of all facilities, and it fully complied with the provisions of the Declaration of Helsinki. Informed consent was obtained from all individual participants.

Research design and treatment methods

This study is a multicenter, open-label, single-arm, prospective study. The registration period was February 1 to October 20, 2016. We received informed consent from all participants and then conducted each examination. The study started on July 20, 2017, and followed up until October 20, 2017. The study design is depicted in Figure 1. During the pre-visit prior to the start of administration of the investigational drug, the candidates were presented with written consent documents describing the purpose of the study and the potential risks associated with it. After agreeing to consent, patients were interviewed and underwent various tests to determine their eligibility as

well as for exclusion criteria. Once patients met all the criteria, they were registered for the trial.

Administration of canagliflozin (CANAGLU Tablets 100 mg) to patients was carried out starting the day after visit 1, with one 100-mg dose per day taken before or after breakfast via oral administration. The period of administration was set to 3 months to observe increasing LDL levels until they reach a plateau. Observations and blood tests during the period of investigational drug administration took place at visit 1 (baseline) and visit 2 (end of the study after 3 months of drug administration). At each visit, blood sample was taken for blood glucose, lipids, and blood biochemical testing, and samples were provided to the central measurement institution (for measurement and evaluation under uniform conditions).

Fibrate-related drugs and pioglitazone (Actos) were prohibited during the administration of the investigational drug due to its known effects on lipid profiles. The following patient treatments already being taken were allowed during the study: statins for hyperlipidemia treatment, ezetimibe, resin products, probucol, nicotinic acid derivative, polyunsaturated fatty acid, diabetes drugs such as DPP-4 inhibitor, GLP-1 receptor agonists, biguanides, α-glucosidase inhibitors, sulfonylurea drugs, fast acting insulin secretory drugs, and insulin derived drugs. However, they were not allowed to change the usage and dosage as well as be prescribed new drugs.

Measurements for evaluation

The background of registered patients including age (date of birth), sex, height, weight, body mass index (BMI), presence or absence of comorbid hyperlipidemia, and allergies for SGLT2 inhibitors were evaluated during the pre-visit (at the time of registration).

Measurements made for efficacy study at pre-visit, visit 1 (start of administration), and visit 2 (3 months after the start) included HbA1c as a blood glucose evaluation item, 20 detailed lipoprotein fractions (chylomicron-cholesterol [CM-C]), very-low-density lipoprotein–cholesterol (VLDL-C), LDL-C, HDL-C, large HDL–cholesterol (LHDL-C), very large HDL–cholesterol (VLHDL-C), small dense LDL–cholesterol (sd-LDL-C), TC, TG, and non-HDL cholesterol (calculated value) as lipid-related items, body weight, and BMI. The 20 detailed lipoprotein fractions were measured at Skylight Biotech Analysis Center (Akita, Japan) using Lipo SEARCH high sensitivity gel filtration HPLC method.

Measurements made for safety study at pre-visit, visit 1, and visit 2 included subjective and objective symptoms, vital signs (systolic and diastolic blood pressure, heart rate at sitting position), and blood biochemical tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], blood urea nitrogen [BUN], γ-glutamyl transpeptidase [γGTP], creatine kinase [CK], uric acid, creatinine [Cre], BUN, eGFR [calculated values], Apo-B, LDL-C). Adverse events, their symptoms, and severity were monitored throughout the study period.

To evaluate the efficacy of the drug, the following 3 points were used as primary measurements: (1) measurements of 20 detailed lipoprotein fractions and their changes in magnitude and ratio from baseline, (2) measurements of blood glucose–related items and their change in magnitude and ratio from baseline, and (3) measurements of weight and BMI and their change in ratio from baseline.

To evaluate the safety of the drug, the following 3 points were used as sub-primary measurements: (1) measurement of clinical tests (blood biochemical tests) and their changes in magnitude and ratio from baseline, (2) vital measurements and their changes in magnitude and ratio from baseline, and (3) adverse events raised between baseline and visit 2.

Statistical analysis

We evaluated the effects of canagliflozin administration on 20 detailed lipoprotein fractions (cholesterol percentage, particle number), blood glucose (HbA1c) values, body weight, and BMI. The changes in magnitude and ratio from baseline for 20 detailed lipoprotein fractions, HbA1c, body weight, and BMI were determined and statistical significance was evaluated by paired *t*-test analyses. The changes in magnitude and ratio were defined as below:

Magnitude of change = value after 3 months of starting drug administration – baseline value

Ratio of change = magnitude of change / baseline value × 100 (%)

Statistical software R (Ver.3.2.4) was used for statistical analysis with a P value $< .05$ being considered significant.

Results

Participant background

The background of study participants is shown in Table 1.

Patients consisted of 13 men (59%) and 9 women (41%) with an age of 60.7 ± 11.6, weight 70.0 ± 11.5 kg, and BMI 25.8 ± 3.8. LDL-C values were 121.3 ± 25.3 mg/dL, and the average value showed a value near the upper limit of the normal range. Half of the participants (11 patients) had a history of hyperlipidemia and 6 patients were using statins. HbA1c value was 8.5% ± 1.1%. The usage of diabetes drugs included insulin (1 patient), SU drugs (4 patients), DPP4 inhibitors (16 patients), and other oral drugs (7 patients). There was no difference in sex between the patient background.

Changes in body weight, BMI, and HbA1c

The changes in clinical data are shown in Table 2. After canagliflozin administration, body weight was significantly reduced from 69.7 to 67.9 kg ($P < .001$). In parallel with this, BMI decreased from 25.7 to 25.0 ($P < .001$). Furthermore, HbA1c

Table 1. Characteristics of study participants.

BACKGROUND	ALL (N = 22)
Age (years)	59.5 ± 12.2
BMI	26.0 ± 3.9
SBP (mm Hg)	125.9 ± 16.8
DBP (mm Hg)	78.2 ± 9.0
HbA1c (NGSP)	8.3 ± 1.1
eGFR (mL/min/1.73 m2)	84.1 ± 18.8
LDL-C (mg/dL)	119.8 ± 25.3
History of hyperlipidemia	12 (48%)
The usage of insulin	2 (8%)
The usage of SU drugs	5 (20%)
The usage of DPP4 inhibitors	16 (64%)
The usage of other oral diabetes drugs	10 (40%)
The usage of statin	7 (28%)

Values expressed as mean ± SD, or number (percent).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; SU, sulfonylurea.
*$P < .05$; ***$P < .001$ between groups.

was significantly reduced from 8.5% to 7.4% ($P < .001$), indicating an improvement of blood glucose levels by the SGLT2 inhibition effects of canagliflozin.

Lipid data and changes in lipoprotein fractions

Lipid data and changes in the main lipoprotein fractions taken before and after canagliflozin administration (visit 1 to visit 2) were shown in Table 3, respectively. In addition, changes in 20 detailed lipoprotein fractions with particle numbers (nM) of each fraction were shown in Table 4 and Figures 2 and 3.

Lipid data (Table 3) showed no significant changes in the various cholesterol values measured after 12 weeks of canagliflozin administration including TG, TC, Apo-B, and non-HDL cholesterol. Similarly, 4 main lipoprotein fractions did not show changes in various cholesterol values in CM, VLDL, LDL, and HDL as well as LDL/HDL. The detailed lipoprotein fractions (Table 4, Figures 2 and 3) showed significant increases in cholesterol values in VLHDL (the 20 detailed lipoprotein fractions; total of G14, G15), LHDL (G16), with an increase of 10.9, 11.5%, respectively ($P < .05$). Furthermore, particle numbers were significantly increased in fraction VLHDL by 10.0% ($P < .05$). There was no significant change in the other fractions such as S-LDL (the 20 detailed lipoprotein fractions; total of G10, G11, G12, G13) or VS-LDL (same as previous, total of G11, G12, G13).

Safety

Adverse events raised after drug administration in this study were 1 patient for common cold, 3 patients for skin surface and skin abnormalities (acronyx, rash, vulval candidiasis), and 1 patient for stroke. Common cold and acronyx were determined to be adventitious and did not correlate with the investigational drug. Correlations between the investigational drug and rash and vulval candidiasis were not denied. Stroke in this case was determined to be non-serious, and because the patients have underlying arterial sclerosis, the event was determined to have less correlation with the investigational drug.

Safety parameters were shown on Table 2. As indicated, there was a significant decrease in the vital sign shift in systolic blood pressure from 129.3 to 119.5 mm Hg ($P = .008$). Furthermore, clinical blood test showed shifts with a significant decrease in values of ALT, ALP, and γGTP and a significant increase in BUN and Cre. However, the changes in values were all within normal ranges. eGFR also showed a significant decrease; however, the difference was small (80.2 to 76.5 mL/min/1.73 m^2).

Discussion

There are reports that pharmacologic drugs for hyperlipemia reduce risks for cardiovascular events, and studies have detected changes in lipid profiles before and after administration of fibrate drugs. A study of long-term administration of the fibrate drugs on patients with type 2 diabetes resulted in a significant reduction of cardiovascular event risks compared with the placebo group. In addition, the effects of fibrate drugs on decreases in TG are thought to activate lipoprotein lipase-mediated promotion of very-low-density lipoprotein-1 (VLDL1) catabolic reactions. This leads to LDL-C to grow in size and reduces small dense LDL (sd-LDL) volume.[11] It has been reported that the decreased sd-LDL volume leads to a reduction in cardiovascular event risks regardless of the increase or decrease in enlarged LDL, suggesting sd-LDL is a risk factor for cardiovascular events.[6] Enlarged LDL-C loses the ability to invade vascular subendothelium, which increases its affinity for liver LDL receptors thereby enhancing the metabolism of LDL-C uptake. Therefore, we believe LDL will decrease in the long term even with short-term accumulation of enlarged LDL. Based on the above, we believe the reduction of the volume or changes in the quality of sd-LDL will lead to a reduction in cardiovascular event risks.[12] It was also reported that higher large HDL-C but not medium, small, or total HDL-C is associated with lower cardiovascular risk in a prospective cohort study in 591 patients with stable CAD. Salonen et al[13] reported that large HDL-C levels (HDL2-C) were inversely associated with the risk of acute myocardial infarction and may thus be protective factors with an analysis involving 1799 patients. Similarly, in the Quebec Cardiovascular Study covering 1169 French-Canadian men younger than 60 years, large HDL-C (HDL2-C) but not small HDL-C (HDL3-C) was inversely correlated with the incidence of ischemic heart disease.[14]

Table 2. Changes in BW, BMI, HbA1c, vital signs, and laboratory data before and after administration of canagliflozin.

	PRE	POST	RATIO	*P*
BW (kg)	**69.7 ± 11.6**	**67.9 ± 11.7**	**−2.6**	**<.001*** **
BMI	**25.7 ± 3.7**	**25.0 ± 3.8**	**−2.7**	**<.001*** **
HbA1c (%)	**8.5 ± 1.2**	**7.4 ± 0.7**	**−12.9**	**<.001*** **
SBP (mm Hg)	**129 ± 9.8**	**119 ± 11.7**	**−7.6**	**.008***
DBP (mm Hg)	75.1 ± 5.7	72.4 ± 8.1	−3.6	.265
HR (bpm)	71.7 ± 11.4	73.0 ± 17.2	1.8	.671
AST (IU/L)	21.8 ± 6.4	21.0 ± 5.5	−3.7	.283
ALT (IU/L)	**28.6 ± 18.5**	**24.6 ± 15.7**	**−14**	**.016***
ALP (IU/L)	**244 ± 54.3**	**224 ± 51.3**	**−8.2**	**.003***
γGTP (IU/L)	**44.6 ± 32.0**	**37.7 ± 26.0**	**−15.5**	**.013***
CK (IU/L)	100 ± 37.9	100 ± 69.5	0.5	.964
BUN (mg/dL)	**14.6 ± 2.1**	**16.8 ± 3.8**	**15.1**	**.001***
Cr (mg/dL)	**0.73 ± 0.2**	**0.78 ± 0.2**	**6.9**	**.006***
eGFR (mL/min/1.73 m2)	**80.2 ± 19.2**	**76.5 ± 22.0**	**−4.8**	**.034***
Uric acid (mg/dL)	5.6 ± 1.5	5.1 ± 1.7	−8.9	.064

Values expressed as mean ± SD, or number (percent).
Abbreviations: BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; HR, heart rate; Post, post-treatment; Pre, pre-treatment; Ratio, ratio of change (%); SBP, systolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; γGTP, γ-glutamyl transpeptidase; CK, creatine kinase.
Values with significant differences before and after administration of canagliflozin are marked in bold.
*$P < .05$; **$P < .01$; ***$P < .001$ vs pre-treatment.

Table 3. Changes in lipid data before and after administration of canagliflozin.

	PRE (MG/DL)	POST (MG/DL)	RATIO	*P*
TG	154 ± 61.2	139 ± 51.0	−9.3	.180
TC	180 ± 23.5	188 ± 32.5	4.7	.079
VLDL	133 ± 45.9	122 ± 43.5	−8.5	.188
LDL	121 ± 23.8	125 ± 25.7	4.0	.203
Apo-B	99.5 ± 17.5	100 ± 19.9	1.4	.562
HDL	59.8 ± 11.8	62.7 ± 14.2	4.9	.062
CM	18.5 ± 19.5	16.6 ± 14.5	−10.1	.592
Non-HDL	120 ± 23.7	125 ± 31.0	4.6	.125
LDL/HDL	2.1 ± 0.6	2.1 ± 0.6	0	.952

Values expressed as mean ± SD, or number (percent).
Abbreviations: Apo-B, apoprotein B; CM: chylomicron; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Post, post-treatment; Pre, pre-treatment; Ratio, ratio of change (%); TC, total cholesterol; TG, triglyceride; VLDL, very-low-density lipoprotein.
*$P < .05$ vs pre-treatment.

Therefore, it has been considered that the cholesterol concentration in specific HDL subpopulations may be more valuable than the TC contained in HDL.

Previous reports using empagliflozin[1] have demonstrated reductions in cardiovascular event risks by a type 2 diabetes drug SGLT2 inhibitor, and more recently, canagliflozin was also found to inhibit cardiovascular and renal events (CANVAS trial).[15]

As mentioned above, SGLT2 inhibitors such as canagliflozin can affect lipid profiles. At the time of drug development as treatment of type 2 diabetes, increases in LDL-C were observed and it was thought to cause compensatory enhancement of lipid metabolism due to inhibition of blood glucose reabsorption, leading to secondary increases in LDL-C levels. However, the mechanisms as to why the administration of SGLT2 inhibitors alters LDL-C particle size remain unknown. Therefore, it is clinically important to examine particle size or effects on subfractions of LDL or HDL, in patients who were administered SGLT2 inhibitors and their correlation with reductions in cardiovascular event risks.

We should also consider the hypothesis that the changes in lipoproteins with canagliflozin are simply due to improvement

Table 4. Changes in details of lipoprotein subfractions before and after administration of canagliflozin.

	PRE (MG/DL)	POST (MG/DL)	RATIO	*P*	PRE (NM)	POST (NM)	RATIO	*P*
L-VLDL	19.8 ± 5.8	20.6 ± 8.3	4.0	.607	54.9 ± 19.4	51.3 ± 19.2	6.6	.381
M-VLDL	8.52 ± 2.5	8.50 ± 2.4	−0.2	.955	48.7 ± 14.6	45.6 ± 13.5	−6.3	.110
S-VLDL	6.67 ± 2.1	6.68 ± 2.1	0.2	.965	47.3 ± 13.1	45.9 ± 12.8	−2.9	.544
L-LDL	23.7 ± 5.5	24.7 ± 7.1	4.3	.305	201 ± 43.2	207 ± 54.2	2.7	.478
M-LDL	41.6 ± 8.1	43.9 ± 10.6	5.5	.159	536 ± 101	516 ± 128	−3.7	.190
S-LDL	21.5 ± 5.8	22.8 ± 5.9	5.9	.137	322 ± 84.0	388 ± 82.5	20.8	.160
VS-LDL	8.67 ± 2.3	9.2 ± 2.4	6.5	.078	177 ± 45.4	187 ± 44.5	5.7	.099
VL-HDL	**2.29 ± 0.9**	**2.54 ± 1.1**	**10.9**	**.003****	**180 ± 70.0**	**198 ± 87.8**	**10.0**	**.030***
L-HDL	**7.38 ± 4.7**	**8.23 ± 5.4**	**11.5**	**.034***	1041 ± 640	1152 ± 736	10.6	.052
M-HDL	15.9 ± 4.1	16.9 ± 4.6	6.3	.081	3620 ± 804	3818 ± 961	5.5	.089
S-HDL	14.2 ± 1.8	14.5 ± 2.0	2.3	.327	14.4 ± 2.2	14.9 ± 2.3	3.5	.466
VS-HDL	6.49 ± 0.8	6.67 ± 1.0	2.8	.247	6.43 ± 0.9	6.65 ± 1.2	3.4	.280

Values expressed as mean ± SD, or number (percent). L-VLDL = G3 +G4 + G5, M-VLDL = G6, S-VLDL = G7, L-LDL = G8, M-LDL = G9, S-LDL = G10, VS-LDL = G11 + G12 + G13, VL-HDL = G14 + G15, L-HDL = G16, M-HDL = G17, S-HDL = G18, VS-HDL = G19 + G20.
Abbreviations: HDL, high-density lipoprotein; L, large; LDL, low-density lipoprotein; M, medium; Post, post-treatment; Pre, pre-treatment; Ratio, Ratio of change (%); S, small; VL, very large; VLDL, very-low-density lipoprotein; VS, very small.
Values with significant differences before and after administration of canagliflozin are marked in bold.
*$P < .05$; **$P < .01$ vs pre-treatment.

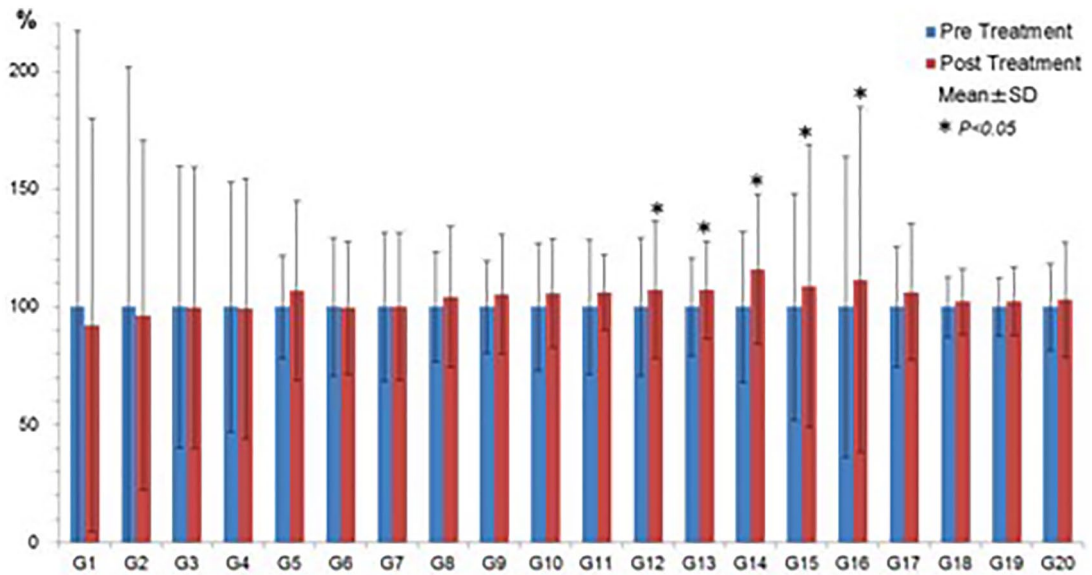

Figure 2. Change rate of lipoprotein details 20 fractions before and after administration of canagliflozin: The rate of change in post-treatment (red bars) compared with pre-treatment (blue bars) in each lipid fraction is shown.

in diabetic control. In type 2 diabetes, there is TG-enrichment of HDL particles; overactive hepatic lipase degrades the TG in those HDL particles, so the HDL particle reduced size and was eliminated through the kidney.[16] Another hypothesis is that canagliflozin improves diabetic control, there is no degradation of HDL particles, and thus there is no reduction in the size of HDL particles, which could explain the increase in very large HDL particles in this study.

There were no significant changes in lipid data (TG, TC, Apo-B), cholesterol in 4 main lipoprotein fractions (CM, VLDL, LDL, HDL), cholesterol in S-LDL, and VS-LDL cholesterol values of 22 patients in the full analysis set (FAS) group before and after canagliflozin administration. Previous reports have consistently found that SGLT2 inhibitors increase both LDL and HDL while slightly reducing TG.[17] In our study, patients have a normal lipid profile (TG 154, HDL 60;

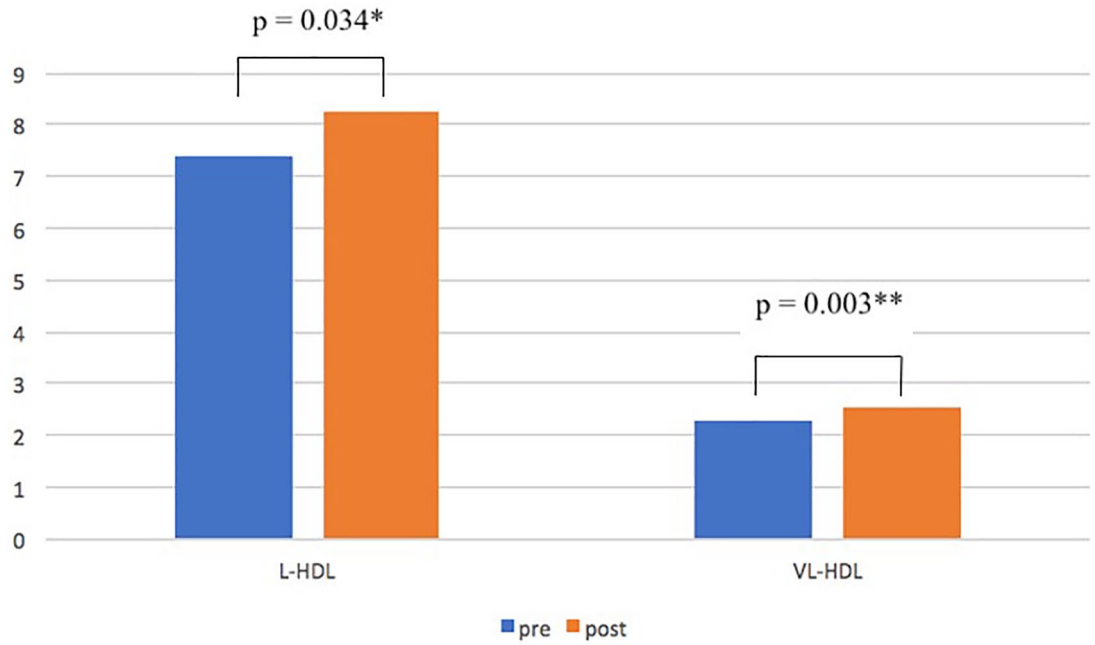

Figure 3. Change of L-HDL and VL-HDL before and after administration of canagliflozin: The change in post-treatment compared with pre-treatment in each lipid fraction is shown. L-HDL increased from 7.38 ± 4.7 to 8.23 ± 5.4, VL-HDL increased from 2.29 ± 0.9 to 2.54 ± 1.1. L-HDL indicates large high-density lipoprotein; VL-HDL, very-large high-density lipoprotein.

while conventional T2DM tend to have higher TG and lower HDL) and perhaps that is 1 reason of why Cana had no effect on lipids.

Cholesterol in the 20 detailed lipoprotein fraction (G1-G20) subclasses showed significant increases in the VL-HDL fractions (G14, G15) and L-HDL (G16) fractions. Furthermore, the VL-HDL fraction showed a significant increase in particle numbers (unit = nM). Significant improvements in blood glucose levels (HbA1c) and reduced body weight by the SGLT2 inhibitory action of canagliflozin are also shown.

In this study, we did not see a significant decrease in S-LDL (G10, G11, G12, G13), which is a factor for reducing risks associated with cardiovascular events. Furthermore, we did not make any observations that suggest enlargement of LDL. It was reported that dapagliflozin increased LDL, but decreased S-LDL,[18] and there was possibly a difference between formulations of SGLT2 inhibitors and measurement methods. However, even for the same class of diabetes drugs, effects other than hypoglycemic effects should be carefully considered individually. For example, in CANVAS study, although the amputation increased with canagliflozin in the CANVAS study, there was no increase in lower limb amputation in the investigation of empagliflozin reported later.[19] There is also a report suggesting the possibility that canagliflozin may reduce gastrointestinal cancer which is not seen in other SGLT2 inhibitors.[20] One reason of these results is the difference in specificity to SGLT2.[21] Based on these arguments, effects other than hypoglycemic effects in diabetes treatment drugs are not necessarily "Class Effect" and should be verified individually.

According to the result of our study, the reduction in risks in cardiovascular events (cardiovascular mortality, non-fatal myocardial infarction, and non-fatal stroke) seen with SGLT2 inhibitors may be due to mechanisms different from those of fibrate drugs that are known to reduce S-LDL volume. However, changes in the subclasses of VL-HDL and L-HDL in the detailed lipoprotein fractions suggested the possibility of inverse association with cardiovascular risk during the development of canagliflozin.

Our study has a number of strengths. It is the first study to evaluate the effects of canagliflizon on lipid profiles and to show the increase effect on VL-HDL and L-HDL. In addition, there are few reports on detailed lipid 20 fraction using HPLC method with SGLT2 inhibitor. Nevertheless, we have some limitations. First, as this study is a single-arm study, it is susceptible to confounding factors. However, in this multi-center research, prescribers, research planners, and practitioners and analysts were separated, decreasing selective bias and possible confounding factors. In the future, it will be necessary to have a larger crossover comparison test or randomized comparison test. Second, it was a negative study that did not give the same results as other SGLT2 inhibitors. Certainly, it may not be consistent between SGLT2 inhibitors, but it does not necessarily have a class effect. Rather, the difference in outcomes among SGLT2 inhibitors would be worth noting.

To conclude, we showed a change in cholesterol levels in the 20 lipoprotein fractions (an increase in very large HDL [G14, G15], large HDL [G16]) after 12 weeks of canagliflozin treatment. Further studies should recommend deepening discussion

on the differences in drug effects as well as class effects in SGLT2 inhibitors.

Author Contributions

Research design was performed by YK, HI, EK, and MY; data acquisition by HI, TY, KK, HA, SM; and data analysis/interpretation by YK, EK. The research director was MY. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Ethical Approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study. This study was approved by the ethics committee of the Medical Hospital, Tokyo Medical and Dental University, approved on December 15, 2016 (No. R2015-501). Clinical research identification number was UMIN000020027.

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