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Improvement of postprandial hyperglycemia and arterial stiffness upon switching from premixed human insulin 30/70 to biphasic insulin aspart 30/70

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Abstract

Postprandial hyperglycemia is known to be associated with increasing cardiovascular mortality in type 2 diabetes mellitus patients. Cardio-ankle vascular index (CAVI) reflects arterial stiffness and is more useful for predicting coronary atherosclerosis than intima-media thickness. Premixed human insulin 30/70 (BHI30) containing rapid-acting insulin has been used conventionally as a biphasic insulin. Recently, a biphasic insulin analogue preparation, biphasic insulin aspart 30/70 (BIAsp30), containing ultrarapid-acting insulin has been approved and expected to improve postprandial hyperglycemia. The aim of this study was to clarify the effects of switching the biphasic insulin from BHI30 to BIAsp30 on arterial stiffness in type 2 diabetes mellitus patients. Twenty-six type 2 diabetes mellitus patients (glycosylated hemoglobin >6.5%) who were already receiving biphasic insulin therapy with BHI30 twice daily were observed for 3 months. Afterward, BHI30 was switched to BIAsp30. At 3 months after switching, relative mobility of the peak of LDL fraction decreased significantly (from 0.3462 ± 0.041 to 0.3356 ± 0.035, $P < .01$); and CAVI also decreased significantly (from 9.77 ± 1.11 to 9.35 ± 1.17 m/s, $P < .005$). A significant negative correlation was observed between the change in CAVI and change in 1,5-anhydroglucitol (1,5-AG) ($r = -0.3929$, $P < .05$). A stronger correlation between change in CAVI and change in 1,5-AG was observed in the subgroup of patients whose 1,5-AG levels were elevated after switching ($r = -0.6261$, $P < .05$) compared with all subjects. These results suggest that switching biphasic insulin from BHI30 to BIAsp30 improves arterial stiffness, and the improvement of arterial stiffness may be associated with improvement of postprandial hyperglycemia.

1. Introduction

Cardiovascular disease is the leading cause of death among type 2 diabetes mellitus patients [1]. Epidemiologic and large-scale intervention studies suggest that postprandial hyperglycemia is associated with increased cardiovascular mortality in type 2 diabetes mellitus patients [2-4]. Furthermore, postprandial hyperglycemia may be an independent risk factor of cardiovascular disease beyond and more powerful than fasting hyperglycemia [5]. Postprandial hyperglycemia and/or concomitant hypertriglycemia may induce endothelial dysfunction and inflammation, and play an important role in the progression of unstable plaque and atherosclerotic disease [6]. However, the mechanism remains unclear.

Acarbose (an α-glucosidase inhibitor), nateglinide, and repaglinide, which improve postprandial hyperglycemia, have been shown to reduce common carotid artery intima-media thickness (IMT) in type 2 diabetes mellitus patients [7-9]. α-Glucosidase inhibitors (α-GIs) also increase serum adiponectin level in patients with type 2 diabetes mellitus [10,11]. Adiponectin is a well-known adipokine that plays a

The study was approved by the institutional review board, and all patients provided written informed consent prior to participation in the study.

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protective role in vascular inflammation and atherosclerosis development [12]. It is possible that improvement of postprandial hyperglycemia protects against atherosclerosis through increasing adiponectin.

Other factors also have relationship with cardiovascular disease or atherosclerosis. Oxidized low-density lipoproteins (LDLs), as modified LDLs, are thought to play a key role in the progression of atherosclerosis [13-15]. Malondialdehyde-modified LDL (MDA-LDL) is one of the oxidized LDLs. MDA-LDL increases in patients with diabetes or hypertriglyceridemia [16]. Elevated levels of MDA-LDL are associated with acute coronary syndrome [17]. High plasma concentrations of remnant-like particles (RLPs), which are formed when triglyceride (TG)-rich lipoprotein are partly depleted of TG by lipoprotein lipase, are considered to be highly atherogenic [18,19]. It has been suggested that plasma RLP-cholesterol (RLP-C) is an independent risk factor for the development of cardiovascular disease [20,21]. The production of vascular reactive oxygen species has been considered to be one of the common pathogenic factors of vascular complication [22,23]. Intracellular reactive oxygen species can cause strand breaks in DNA and base modifications, including the oxidation of guanine residues to 8-hydroxydeoxyguanosine (8-OHdG) [24]. Small-sized LDL particles are well known to be atherogenic [25,26], and normalization of LDL particle size is one of the antiatherogenic changes. High urinary albumin is a risk factor of cardiovascular disease in patients with type 2 diabetes mellitus [27].

Recently, an arterial stiffness parameter called *cardio-ankle vascular index* (CAVI) has been developed as a marker of arteriosclerosis including the aorta, femoral artery, and tibial artery [28]. Cardio-ankle vascular index is measured from an electrocardiogram, phonocardiogram, brachial artery waveform, and ankle artery waveform, and is adjusted for blood pressure based on the stiffness parameter β [29]. It is independent of blood pressure and has adequate reproducibility for clinical use [28]. Furthermore, no special technique is required for the measurement of CAVI. Arterial stiffness can be evaluated by measuring brachial-ankle pulse wave velocity (PWV) and CAVI. Cardio-ankle vascular index is superior to brachial-ankle PWV as an index of arterial stiffness in patients who have undergone coronary angiography [29]. Several reports have shown that CAVI is useful for the detection of atherosclerotic diseases [28,30,31] and is superior to IMT for predicting coronary atherosclerosis [32]. It is possible that high CAVI reflects progression of atherosclerosis, especially coronary atherosclerosis.

Rapid-acting insulin formulations have been used clinically for a long time. These conventional formulations are injected 30 minutes before meals and maintain a blood insulin concentration profile that mimics the dynamics of insulin secretion occurring postprandially in healthy individuals. Premixed human insulin 30/70 (BHI30) is mixed 30% rapid-acting human insulin and 70% neutral protamine Hagedorn. Insulin aspart and insulin lispro are 2 ultrarapid-acting insulin analogues that were developed and approved for clinical use in the 1990s. A biphasic insulin analogue preparation, biphasic insulin aspart 30/70 (BIAsp30), was approved and became available overseas in 2000 and in Japan in 2003. This biphasic insulin analogue consists of 30% free and 70% protamine-bound insulin aspart; and twice-daily premeal injection of this preparation is expected to improve postprandial hyperglycemia, thus potentially reducing the risk for cardiovascular disease.

We hypothesized that switching the biphasic insulin from BHI30 to BIAsp30 improves postprandial hyperglycemia. Improving of postprandial hyperglycemia leads to decrease MDA-LDL, RLP-C, LDL, and urinary albumin; increased serum adiponectin; and enlarged LDL particle size. As a result, CAVI, which is one of the surrogate parameters of cardiovascular disease, is decreased. This study was conducted to evaluate the effect of switching the biphasic insulin from BHI30 to BIAsp30 on postprandial hyperglycemia and arterial stiffness.

2. Subjects and methods

2.1. Subjects

This study was approved by the institutional review board. Before participation, the purpose of this study was explained to each subject; and consent was obtained for participation in the study and also for release of the study data.

Twenty-six patients with type 2 diabetes mellitus (glycosylated hemoglobin [HbA_{1c}] >6.5%) who were already receiving insulin therapy with BHI30 twice daily were enrolled. All patients injected BHI30 30 minutes before breakfast and dinner. Afterward, the conventional biphasic human insulin BHI30 was changed to the biphasic insulin analogue BIAsp30 and was injected immediately before breakfast and dinner. The amount of insulin BIAsp30 was the same as insulin BHI30. Table 1 shows the clinical characteristics of the subjects at baseline. The subjects were observed for 3 months after the switch of biphasic insulin. We measured the body mass index (BMI), fasting blood glucose (FBG), HbA_{1c}, 1,5-anhydroglucitol (1,5-AG), serum total cholesterol (TC) levels, serum TG levels, serum high-density lipoprotein cholesterol (HDL-C) levels, serum LDL-cholesterol (LDL-C) levels, serum adiponectin, MDA-LDL, RLP-C, and urinary 8-OHdG every month for 3 months in this study. The LDL particle size and CAVI were measured before and after 3 months. During this study, all patients maintained the same diet and exercise therapies and did not change medications. All subjects received nutritional guidance from a dietitian every month. The dietitian analyzed the meals of the patient and suggested changes if necessary.

2.2. Measurement of body weight and blood sampling

Body weight was measured and blood samples were collected in the morning after 12 hours of fasting. Serum was

Table 1
Baseline characteristics and changes in various parameters after switching from BHI30 to BIAsp30

	Baseline	Time after switching		
		1 mo	2 mo	3 mo
No. of subjects (male/female)	26(12/14)			
Age (y)	64.58 ± 9.47			
BW (kg)	56.09 ± 10.33	5624 ± 9.99	56.40 ± 9.65	56.68 ± 9.90
BMI (kg/m^2)	22.63 ± 3.27	22.69 ± 3.19	22.77 ± 3.10	22.87 ± 3.13
FBG (mg/dL)	154.61 ± 75.68	152.50 ± 62.80	147.00 ± 60.94	153.65 ± 66.40
HbA_{1c} (%)	7.51 ± 1.29	7.57 ± 1.23	7.63 ± 1.20	7.50 ± 1.11
1,5-AG (μg/mL)	6.84 ± 5.07	6.55 ± 5.73	6.99 ± 5.23	6.99 ± 5.29
TC (mg/dL)	189.58 ± 32.14	194.11 ± 33.41	195.15 ± 33.90	197.23 ± 39.49
TG (mg/dL)	120.92 ± 70.15	134.15 ± 82.58	154.65 ± 130.13	152.12 ± 110.70
HDL-C (mg/dL)	58.35 ± 18.65	56.73 ± 19.37	57.19 ± 20.23	59.58 ± 19.72
LDL-C(mg/dL)	104.00 ± 29.96	106.96 ± 29.13	105.81 ± 32.61	105.69 ± 31.64
Adiponectin (ng/mL)	12.87 ± 7.77	12.08 ± 6.73	12.97 ± 8.14	13.10 ± 834
MDA-LDL (U/L)	104.00 ± 74.18	106.58 ± 75.19	103.13 ± 53.08	106.90 ± 80.01
RLP-C (mg/dL)	5.97 ± 4.97	7.02 ± 6.01	845 ± 10.64	7.19 ± 625
Urinary albumin (mg/g Cr)	680.34 ± 1618.38	470.10 ± 968.79	556.72 ± 1161.51	586.22 ± 1227.07
Urinary 8-OHdG (ng/mg Cr)	8.30 ± 3.38	8.77 ± 2.65	7.97 ± 2.55	7.36 ± 3.37
LDL-Rm ratio	0.3462 ± 0.041			0.3356 ± 0.035*

Data are presented as mean ± SD. BW indicates body weight.
* $P < .01$ vs control.

separated within 1 hour; and the sample was used for measurements of HbA_{1c}, 1,5-AG, and serum lipids, and analysis of serum lipoproteins by polyacrylamide gel disc electrophoresis and adiponectin.

2.3. Measurement of HbA_{1c} and plasma lipid concentrations

For measuring HbA_{1c}, the blood was collected in tubes containing EDTA. Glycosylated hemoglobin including stable and unstable fractions was measured by the high-pressure liquid chromatography method using Hi-Auto A_{1c} (Kyoto Daiichi Kagaku, Kyoto, Japan). The data of the stable form were used in the present analysis.

Serum concentration of 1,5-AG was measured by an enzymatic method using Determina-L 1,5-AG (Kyowa Medics, Tokyo, Japan; normal >14 mg/mL). Some studies found 1,5-AG to generally reflect postprandial hyperglycemia [33-36]. 1,5-AG reflects glycemic excursions, often in the postprandial state, more robustly than fructosamine or HbA_{1c} [34]. 1,5-Anhydroglucitol also reflects 2-hour postprandial glucose in outpatients [37].

Plasma TC, TG, and LDL-C levels were measured enzymatically using kits from Nippon Shoji (Osaka, Japan) and a HITACHI 7150 analyzer (HITACHI, Tokyo, Japan). High-density lipoprotein cholesterol was measured by a selective inhibition method (Daiichi Pure Chemicals, Tokyo, Japan) [38].

2.4. Measurement of serum adiponectin

Serum adiponectin level was measured using an enzyme-linked immunosorbent assay (ELISA) system (adiponectin ELISA kit; Otsuka Pharmaceutical, Tokushima, Japan), as reported previously [39].

2.5. Measurement of MDA-LDL

The ELISA used for measurement of MDA-LDL was based on the method reported by Kotani et al [40]. In brief, microtiter plates were coated with a monoclonal antibody against MDA-LDL. Duplicate 100-mL samples were added to the wells of the plates and incubated for 1 hour at room temperature. After washing, a b-galactosidase–conjugated monoclonal antibody against apolipoprotein B was added to each well; and the mixture was incubated for 1 hour at room temperature. After washing, 100 mL of the substrate solution, *o*-nitrophenyl-b-galactopyranoside (10 mmol/L), was dispensed into each well and allowed to react for 2 hours at room temperature. The reaction was terminated by adding a stop solution, and absorbance was determined spectrophotometrically at 415 nm. The primary standard used was artificially prepared MDA-LDL in which 15% of the amino groups were modified. From a curve constructed using this standard, the amount of MDA-LDL in the samples was determined. One unit per liter was defined as the absorbance obtained with the standard at a concentration of 1 mg/L.

2.6. Measurement of RLP-C

Remnant-like particle cholesterol was measured using the MetaboLead RemL-C kit (Kyowa Medex, Tokyo, Japan) and analyzed by an automated analyzer (BM1650; JEOL, Tokyo, Japan).

2.7. Urinary albumin analysis

Urinary albumin concentration was determined by turbidimetric immunoassay using the Superior-Microalbumin kit (Mitsubishi Chemical Medience, Tokyo, Japan) and was corrected for urinary creatinine (Cr) concentration. Urinary Cr was measured by the enzymatic reaction.

2.8. Urinary 8-OHdG analysis

Urine samples were centrifuged at 800*g* for 10 minutes, and the supernatant was used for the determination of 8-OHdG by a competitive ELISA (8-Hydroxydeoxyguanosine Check; Japan Institute for the Control of Aging, Shizuoka, Japan). The monoclonal antibody has been characterized and found to be specific for 8-OHdG [41]. The result was expressed as per milligram Cr in the urine sample.

2.9. Measurement of LDL particle size

Serum lipoproteins were separated by polyacrylamide gel disc electrophoresis using the Lipo Phor system (Quantimetrix, CA, USA; Jyohkoh, Tokyo, Japan) [42]. Low-density lipoprotein particle size was evaluated using the relative mobility of the peak of LDL fraction (LDL-Rm) obtained from the densitometric pattern (Densitron 20-HR, Jyohkoh). In this method, a decrease in Rm ratio indicates an increase in LDL particle size on polyacrylamide gel disc electrophoresis [43,44].

2.10. Measurement of CAVI

Cardio-ankle vascular index is obtained by measuring blood pressures and PWV according to the following formula: $CAVI = a\{(2\rho/\Delta P) \times \ln(Ps/Pd)PWV^2\} + b$, where Ps is systolic blood pressure, Pd is diastolic blood pressure, PWV is pulse wave velocity, ΔP is Ps − Pd, ρ is blood density, and a and b are constants. Pulse wave velocity is obtained by dividing the vascular length by the time for the pulse wave to propagate from the aortic valve to the ankle. Pulse waves are measured by cuffs placed at upper arms and ankles; and blood pressure, by cuff at the upper arm. To be compatible with the aortic PWV method established by Hasegawa [45], scale conversion constants (a, b) are determined so as to match CAVI with the aortic PWV method. Using these scale conversion constants, massive previous data of PWV could be converted to CAVI.

In the present study, CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi, Tokyo, Japan) as described previously [28]. Briefly, with the subject supine and the head held in midline position, cuffs were applied to bilateral upper arms and ankles. After resting for 10 minutes, the examination was started. Brachial and ankle pulse waves were measured with the upper arm and ankle cuffs, using a low cuff pressure of 30 to 50 mm Hg to ensure minimal effect of the cuff pressure on hemodynamics. Afterward, blood pressure was measured using the cuff of the upper arm. All the measurements were input into the VaSera, and CAVI was automatically calculated. The average coefficient of variation of CAVI is less than 5%, which is small enough for clinical use and indicates that CAVI has good reproducibility [31].

2.11. Statistical analysis

Data are expressed as mean ± SD. Normal distribution was tested by Shapiro-Wilk test. Some data did not have normal distribution. Normality was secured by a logarithmic transformation. Statistical analysis was performed using repeated-measure analysis of variance. Regression analysis was performed using the JMP computer software (SAS, Cary, NC). P values < .05 were considered significant.

3. Results

3.1. Changes in body weight, BMI, FBG, HbA_{1c}, 1,5-AG levels, and serum lipids after switch of biphasic insulin

Body weight and BMI did not increase after 3 months of switching the biphasic insulin from BHI30 to BIAsp30. Fasting blood glucose and HbA_{1c} were unchanged. The 1,5-AG levels tended to improve after 3 months. Among serum lipids, the changes in TC, TG, HDL-C, and LDL-C were not statistically significant (Table 1).

3.2. Changes in adiponectin, MDA-LDL, RLP-C, urinary albumin, urinary 8-OHdG, and LDL-Rm ratio after switch of biphasic insulin

Adiponectin, MDA-LDL, RLP-C, urinary albumin, and urinary 8-OHdG levels remained unchanged after switch of biphasic insulin. On the other hand, the LDL-Rm ratio decreased significantly from 0.3462 ± 0.041 to 0.3356 ± 0.035 ($P < .01$) after 3 months of switching from BHI30 to BIAsp30 (Table 1).

3.3. Changes in CAVI after switch of biphasic insulin

Cardio-ankle vascular index decreased significantly from 9.77 ± 1.11 to 9.35 ± 1.17 m/s ($P < .005$) after 3 months of switching the biphasic insulin from BHI30 to BIAsp30 (Fig. 1).

3.4. Correlation between decrease of CAVI and changes in metabolic parameters

To clarify the important factors involved in the decrease in CAVI associated with switching biphasic insulin, the

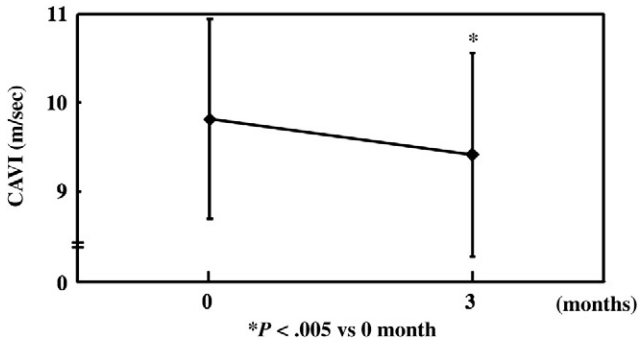

Fig. 1. Change of CAVI after switching biphasic insulin. Data are presented as mean ± SD.

Table 2
Correlation between change in CAVI and changes in various parameters after 3 months of switching biphasic insulin

	Correlation coefficient (r)	P value
ΔBW	0.1863	.3621
ΔBMI	0.1426	.4872
ΔFBG	0.0236	.9090
ΔHbA_{1c}	0.1313	.5227
Δ1,5-AG	−0.3929	<.05
ΔTC	−0.0029	.9888
ΔTG	0.1985	.3317
ΔHDL-C	0.1636	.4246
ΔLDL-C	−0.1511	.4613
ΔAdiponectin	−0.2553	.2081
ΔMDA-LDL	0.1284	.5318
ΔRLP-C	0.1206	.5572
ΔUrinary albumin	−0.1595	.4363
ΔUrinary 8-OHdG	−0.0066	.9745
ΔLDL-Rm ratio	−0.0396	.8479

Correlation coefficient was calculated for change in CAVI ratio vs change in each parameter. Δ means the difference between the value at baseline and the value after 3 months.

correlation between the change in CAVI and changes in various metabolic parameters was analyzed. Among all the factors analyzed, change in 1,5-AG level was the only factor correlated significantly with a decrease in CAVI (Table 2).

3.5. Investigation of 1,5-AG raising group

The above results suggested that decreased CAVI associated with switching of biphasic insulin from BHI30 to BIAsp30 could be mediated thorough a change of 1,5-AG. To examine the relationship between CAVI and 1,5-AG, we divided the patients into 2 subgroup: a subgroup showing increased 1,5-AG after 3 months of switching (1,5-AG elevated group) and a group showing unchanged or decreased 1,5-AG after 3 months (1,5-AG nonelevated group). As shown in Table 3, the baseline data of all parameters did not differ significantly between the 1,5-AG elevated and the 1,5-AG nonelevated groups. In the 1,5-AG elevated group, FBG decreased significantly ($P < .05$) from 154.75 ± 79.59 mg/dL at baseline to 138.33 ± 60.80 mg/dL at 3 months after switching the biphasic insulin, 1,5-AG increased significantly ($P < .005$) from 5.08 ± 3.65 to 6.76 ± 5.23 μg/mL, whereas CAVI decreased significantly from 9.56 ± 1.09 to 9.13 ± 1.06 m/s. All other parameters did not change significantly.

3.6. Correlation between decrease in CAVI and changes in various metabolic parameters in 1,5-AG elevated group

To validate the factors contributing to the decrease of CAVI accompanying switch of biphasic insulin in the 1,5-AG elevated subgroup, the correlation between the change in CAVI and changes in various metabolic parameters was analyzed. Increase in 1,5-AG level was the most important factor significantly related to decreased CAVI (Table 4). The correlation coefficient (−0.6261) was larger than that obtained from the analysis of change in 1,5-AG in all subjects (−0.3929). Change in serum adiponectin level was also a significant factor related to change in CAVI, but the correlation coefficient was smaller than that of 1,5-AG level (Table 4).

Table 3
Baseline characteristics and changes in various parameters when subjects were divided into 1,5-AG elevated and 1,5-AG nonelevated subgroups

	Baseline		After 3 mo	
	1,5-AG elevated subgroup	1,5-AG nonelevated subgroup	1.5-AG elevated subgroup	
No. of subjects (male/female)	12 (4/8)	14 (8/6)		
Age (y)	65.50 ± 11.92	63.79 ± 7.13	NS	
BW (kg)	57.15 ± 13.32	55.18 ± 7.29	NS	57.60 ± 12.68
BMI (kg/m^2)	23.72 ± 4.24	21.69 ± 1.83	NS	23.92 ± 4.04
FBG (mg/dL)	154.75 ± 79.59	154.57 ± 58.48	NS	138.33 ± 60.80*
HbA_{1c} (%)	7.75 ± 1.34	7.31 ± 1.26	NS	7.61 ± 1.19
1,5-AG (μg/mL)	5.08 ± 3.65	8.35 ± 5.74	NS	6.76 ± 5.23†
TC (mg/dL)	185.25 ± 30.12	193.29 ± 34.45	NS	204.50 ± 42.51
TG (mg/dL)	127.42 ± 79.74	115.36 ± 63.33	NS	161.92 ± 101.10
HDL-C (mg/dL)	61.08 ± 21.42	56.00 ± 16.38	NS	63.50 ± 23.10
LDL-C (mg/dL)	93.58 ± 27.72	112.93 ± 29.83	NS	107.00 ± 29.49
Adiponectin (ng/mL)	14 ± 26 ± 10.11	11.69 ± 5.14	NS	15.31 ± 10.20
MDA-LDL (U/L)	109.08 ± 96.81	99.65 ± 51.06	NS	115.68 ± 100.85
RLP-C (mg/dL)	6.27 ± 6.23	5.71 ± 3.82	NS	7.40 ± 5.87
Urinary albumin (mg/g Cr)	454.66 ± 1030.63	873.78 ± 2012.64	NS	712.62 ± 1619.38
Urinary 8-OHdG (ng/mg Cr)	8.60 ± 4.81	8.05 ± 1.50	NS	7.89 ± 3.80
LDL-Rm ratio	0.3392 ± 0.0538	0.3523 ± 0.0275	NS	03348 ± 0.0444
CAVI	9.56 ± 1.09	9.96 ± 1.15	NS	9.13 ± 1.06*

Data are presented as mean ± SD. NS indicates not significant.
* $P < .05$.
† $P < .005$.

Table 4
Correlation between change in CAVI and changes in various metabolic parameter after 3 months in 1,5-AG elevated subgroup

	Correlation coefficient (r)	P value
ΔBW	0.0889	.7836
ΔBMI	0.0004	.9989
ΔFBG	0.0677	.8343
ΔHbA_{1c}	0.2292	.4736
Δ1,5-AG	−0.6261	<.05
ΔTC	−0.0696	.8298
ΔTG	0.2186	.4950
ΔHDL-C	0.4164	.1781
ΔLDL-C	−0.3520	.2618
ΔAdiponectin	−0.4933	<.05
ΔMDA-LDL	−0.0420	.8970
ΔRLP-C	−0.0085	.9792
ΔUrinary albumin	−0.3594	.2513
ΔUrinary 8-OHdG	−0.0591	.8552
ΔLDL-Rm ratio	−0.0179	.9559

Correlation coefficient was calculated for change in CAVI vs change in each parameter. Δ means the difference between the value at baseline and the value after 3 months.

4. Discussion

In this study, LDL particle size was increased and CAVI was decreased in diabetic patients after the biphasic insulin was switched from BHI30 to BIAsp30. The decrease in CAVI correlated with an increase in 1,5-AG. The correlation coefficient between CAVI and 1,5-AG was larger in the subgroup showing elevated 1,5-AG after switching than that in all subjects. In the 1,5-AG elevated subgroup, an increase in serum adiponectin was also related to a decrease of CAVI.

Postprandial hyperglycemia is known to increase cardiovascular mortality in type 2 diabetes mellitus patients [2-4]. Indeed, α-GI, nateglinide, and repaglinide, which improve postprandial hyperglycemia, reduce common carotid artery IMT in type 2 diabetes mellitus patients [7-9]. Coronary heart disease is related to carotid artery IMT [46]. Cardio-ankle vascular index is a useful predictor of coronary atherosclerosis [32] and is increased by postprandial hyperglycemia [47]. In this study, a decrease of CAVI correlated with an increase of 1,5-AG, especially in patients whose 1,5-AG was elevated after switching. This result suggests that improvement of postprandial hyperglycemia by switching the biphasic insulin from BHI30 to BIAsp30 may reduce the risk of coronary heart disease. However, we did not perform coronary angiography. Cardio-ankle vascular index is one of the surrogate parameters of cardiovascular disease. Therefore, it is necessary to perform this consideration carefully.

Some reports show that hypoglycemia may be a risk factor for cardiovascular disease or may increase cardiovascular mortality [48,49]. In this study, postprandial hyperglycemia was decreased; but FBG and HbA_{1c} were not decreased. It is possible that hypoglycemia rarely occurred.

The mechanism of how α-GI or nateglinide reduces IMT remains unclear. Adiponectin, an adipokine that has a protective role against vascular inflammation and development of atherosclerosis [12], is increased by improvement of postprandial hyperglycemia in patients with type 2 diabetes mellitus [10,11]. Adiponectin is also known to decrease C-reactive protein (CRP) synthesis and secretion from endothelial cells [50]. C-reactive protein, the prototypic marker of inflammation in humans, has been shown in several studies to be a cardiovascular risk marker, with high levels of CRP predicting cardiovascular events [51-53]. Our data showed a significant negative correlation between the change in CAVI and change in serum adiponectin in the 1,5-AG elevated subgroup. In other study, CAVI and serum adiponectin level showed negative correlation [54]. It is possible that a decrease in CAVI is associated with an increase in serum adiponectin and a decrease in CRP. However, because we did not measure CRP, the relationship between CAVI and CRP remains unclear in this study. We explain that the investigation of the 1,5-AG raising group is a post hoc analysis.

Atherosclerosis is related to other factors such as oxidative stress and small dense LDL [25,26,55,56]. In this study, we measured urinary 8-OHdG, MDA-LDL, and LDL-Rm ratio. Urinary 8-OHdG and MDA-LDL were unchanged and not related to CAVI. The LDL-Rm ratio, which reflects LDL particle size, was improved in all subjects but did not correlate with CAVI. We hypothesized that these factors improve through decreasing blood glucose in postprandial state, and CAVI is decreased. However, our results could not prove this hypothesis.

This study has 4 limitations. First, only 26 subjects were studied. A previous report showed that BIAsp significantly decreased postprandial plasma glucose compared with BHI30 [57]. In the present study, 1,5-AG level, which reflects the excursion of postprandial glycemia, showed a trend of increase in all subjects; but the change was not significant. This result may be due to the small sample size. Second, the mechanism of decreased CAVI is still unclear. Although serum adiponectin correlated with CAVI, we did not measure CRP or other marker of inflammation. Therefore, we cannot conclude whether serum adiponectin decreases inflammation in arteries. To clarify the effect of switching biphasic insulin from BHI30 to BIAsp30 and to elucidate the detailed mechanisms of the decreased CAVI, further large-scale study is required; and markers of inflammation have to be measured. Third, this study was observational and not blinded or randomized. There were no control subjects in this study. In addition, the investigation of the 1,5-AG raising group is a post hoc analysis. Fourth, it is better to perform continuous glucose monitoring and a 7-point glucose profile to evaluate postprandial glycemic excursions. However, these measurements were not done at all.

In summary, we demonstrated that a switch of biphasic insulin from BHI30 to BIAsp30 in patients with type 2 diabetes mellitus resulted in increased LDL particle size and decreased CAVI. Decrease in CAVI correlated with increase

in 1,5-AG, and this correlation became stronger in patients whose 1,5-AG was elevated after switching. These results suggest that switching biphasic insulin from BHI30 to BIAsp30 improves arterial stiffness, and this effect is associated with improvement of postprandial hyperglycemia and increase of serum adiponectin level.

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