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RESEARCH LETTER

The effect of anagliptin treatment on glucose metabolism and lipid metabolism, and oxidative stress in fasting and postprandial states using a test meal in Japanese men with type 2 diabetes

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It has been generally recognized that postprandial hyperglycemia and hyperlipidemia are highly related to the development of atherosclerosis [1, 2]. Hyperglycemia is known to damage vascular endothelial cells, increase oxidative stress, promote the expression of adhesion molecule and inhibit nitric oxide (NO) production [3]. Remnant lipoprotein, an important component of postprandial hyperlipidemia, promotes foam cell formation of macrophages and proliferation of smooth muscle cells [4]. Dipeptidyl peptidase 4 (DPP-4) inhibitors have attracted attention as a new class of anti-diabetic agents for the treatment of type 2 diabetes [5]. Anagliptin, a member of the medication class of DPP-4 inhibitors, has been recently available in the market in Japan. Animal studies suggest that anagliptin treatment is associated with improvement of glucose tolerance either by amelioration of insulin resistance or enhancing insulin secretion [6] and the decrease in the development of atherosclerosis [7]. However, to our knowledge, there has been no clinical study. In this background, we investigated the effect of anagliptin treatment on glucose and lipoprotein metabolism in fasting and postprandial state using a test meal (JANEF E460F18®, Q.P. Co., Tokyo, Japan).

Ten Japanese men with type 2 diabetes (age 66.3 ± 9.5 years; body mass index (BMI) 26.6 ± 2.2 kg/m^2; waist circumference 94.6 ± 8.0 cm) who had never received any diabetes drugs before they were involved in this study. They were orally administered anagliptin (200 mg per day) for 12 weeks followed by another 12 weeks of discontinuation (at 24 weeks). Postprandial glucose metabolism, lipoprotein metabolism, and oxidative stress markers were evaluated at 0, 12, and 24 weeks of the study period with the test meal according to the protocol we previously reported [8]. Metabolic parameters including lipid, lipoproteins, and oxidative stress markers were measured according to the method we previously reported [8]. Serum apo B-48 levels were measured by the method using a chemiluminescent enzyme assay (Fujirebio Co., Ltd.). High-molecular weight (HMW) adiponectin in plasma was measured by chemiluminescent enzyme assay (Fujirebio Co., Ltd.). Serum lipoprotein lipase (LPL) mass was measured by ELISA (Sekisui Co. Ltd.). This work was carried out in accordance with the Declaration of Helsinki for experiments involving humans. Informed consent was obtained from all of the participants. The institutional review board in Kanazawa Medical University Hospital approved the experimental protocol, and all of the subjects provided informed consent to participate in the study.

Table 1 shows changes in various metabolic parameters at 0, 12, and 24 weeks of the study period. There were slight reductions in BMI of the study subjects at 24 weeks versus 0 week. At 12 weeks of study period, anagliptin treatment was associated with significant reductions in plasma glucose at 0, 60, and 120 min after test meal loading, all of which returned to pretreatment level at

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Table 1 Changes in various metabolic parameters at 0, 12, and 24 weeks of the study period

	0 week	12 weeks	24 weeks	12 weeks vs. 0 week		24 weeks vs. 12 weeks		24 weeks vs. 0 week	
	mean ± STD	mean ± STD	mean ± STD	% change	*P*	% change	*P*	% change	*P*
Age, year	66.30 ± 9.48								
Body mass index, kg/m^2	26.57 ± 2.16	26.24 ± 2.45	25.98 ± 2.46	−0.33	0.0897	-0.27	0.2207	−0.6	0.0522
waist, cm	94.72 ± 7.54	93.05 ± 8.11	94.45 ± 8.01	−1.67	**0.027**	1.4	0.0726	−0.27	0.6935
Plasma glucose, mmol/L									
0 min	7.33 ± 1.24	6.27 ± 0.80	6.64 ± 1.04	−19.20	**0.004**	6.7	0.275	−12.5	**0.016**
60 min	12.03 ± 2.27	9.56 ± 1.90	11.16 ± 2.65	−44.40	**0.002**	28.8	0.131	−15.6	0.105
120 min	10.19 ± 3.33	7.68 ± 1.76	9.56 ± 2.49	−45.10	**0.004**	33.8	**0.01**	−11.3	0.492
Insulin, mU/L									
0 min	8.72 ± 3.02	8.01 ± 4.31	5.73 ± 2.19	−0.71	0.322	−2.28	**0.037**	−2.99	**0.01**
60 min	40.95 ± 14.15	38.97 ± 25.53	32.43 ± 12.30	−1.98	0.557	−6.54	0.695	−8.52	0.232
120 min	37.98 ± 22.03	38.03 ± 25.91	31.96 ± 13.20	0.05	1	−6.07	0.432	−6.02	0.375
HOMA-IR	2.77 ± 0.84	2.20 ± 1.15	1.68 ± 0.68	−0.57	**0.027**	−0.51	**0.049**	−1.08	**0.004**
C-peptide, nmol/L									
0 min	0.73 ± 0.12	0.70 ± 0.14	0.65 ± 0.16	−0.11	0.324	−0.14	0.313	−0.25	0.115
60 min	1.76 ± 0.30	1.83 ± 0.40	1.68 ± 0.35	0.23	0.625	−0.47	0.477	−0.24	**0.477**
120 min	2.04 ± 0.42	2.15 ± 0.51	2.10 ± 0.45	0.36	0.232	−0.17	0.646	0.19	0.826
CPR index	1.73 ± 0.44	1.90 ± 0.45	1.65 ± 0.31	0.17	**0.037**	−0.25	0.064	−0.08	0.625
1,5AG, mmol/L	76.52 ± 46.58	103.20 ± 45.58	82.67 ± 47.40	4.38	**0.008**	−3.37	0.084	1.01	0.375
Glycoalbumin, %	18.12 ± 2.61	15.86 ± 1.46	17.90 ± 2.50	−2.26	**0.002**	2.04	**0.004**	−0.22	0.715
HbA1c, %	7.13 ± 0.70	6.41 ± 0.34	6.82 ± 0.68	−0.72	**0.002**	0.41	**0.035**	−0.31	0.053
Total cholesterol, mmol/L									
0 min	5.07 ± 0.73	4.70 ± 0.79	4.71 ± 0.73	−14.50	**0.025**	0.7	0.846	−13.8	**0.047**
60 min	4.90 ± 0.70	4.52 ± 0.82	4.60 ± 0.76	−14.60	**0.037**	3	0.941	−11.6	0.078
120 min	4.86 ± 0.63	4.52 ± 0.77	4.59 ± 0.74	−13.10	**0.012**	2.4	0.865	−10.7	0.111
[a]Triglycerides, mmol/L									
0 min	1.77 ± 0.98	1.38 ± 0.60	1.40 ± 0.55	−34.40	**0.037**	1.4	0.557	−33	0.064
60 min	2.08 ± 0.94	1.60 ± 0.63	1.77 ± 0.55	−42.80	**0.027**	15.2	0.084	−27.6	0.084
120 min	2.36 ± 1.08	1.69 ± 0.72	2.11 ± 0.82	−58.90	**0.012**	37.2	**0.014**	−21.7	0.322
HDL-cholesterol, mmol/L									
0 min	1.46 ± 0.24	1.43 ± 0.35	1.46 ± 0.32	−1.50	0.375	1.2	0.711	−0.3	0.576
60 min	1.40 ± 0.20	1.38 ± 0.35	1.41 ± 0.32	−0.70	0.344	0.8	0.789	0.1	0.68
120 min	1.39 ± 0.22	1.38 ± 0.34	1.39 ± 0.31	−0.10	0.586	0.4	0.881	0.3	1
Non HDL-cholesterol, mmol/L									
0 min	3.61 ± 0.17	3.27 ± 0.62	3.26 ± 0.63	−13.00	**0.006**	−0.5	0.789	−13.5	**0.039**
60 min	3.50 ± 0.69	3.14 ± 0.62	3.19 ± 0.64	−13.90	**0.004**	2.2	0.77	−11.7	0.074
120 min	3.48 ± 0.62	3.14 ± 0.60	3.19 ± 0.62	−13.00	**0.004**	2	0.82	−11	0.084
LDL-cholesterol, mmol/L									
0 min	2.85 ± 0.50	2.69 ± 0.54	2.55 ± 0.48	−6.40	**0.025**	−5.5	0.287	−11.9	**0.029**
60 min	2.74 ± 0.51	2.53 ± 0.53	2.46 ± 0.47	−8.20	**0.004**	−2.7	0.508	−10.9	**0.027**
120 min	2.71 ± 0.46	2.51 ± 0.48	2.42 ± 0.47	−7.90	**0.008**	−3.3	0.432	−11.2	**0.029**
[a]RLP-cholesterol, mmol/L									
0 min	0.27 ± 0.21	0.21 ± 0.14	0.21 ± 0.13	−2.50	**0.049**	0.26	0.496	−2.24	0.131
120 min	0.33 ± 0.21	0.24 ± 0.15	0.28 ± 0.15	−3.66	**0.02**	1.66	0.064	−2	0.232
Apolipoprotein B, mg/Dl									
0 min	84.90 ± 13.57	77.80 ± 12.22	76.20 ± 12.55	−7.10	**0.035**	−1.6	0.484	−8.7	**0.016**
120 min	80.50 ± 12.09	74.80 ± 12.77	71.30 ± 11.02	−5.70	0.064	−3.5	0.156	−9.2	**0.016**

Table 1 continued

	0 week	12 weeks	24 weeks	12 weeks vs. 0 week		24 weeks vs. 12 weeks		24 weeks vs. 0 week	
	mean ± STD	mean ± STD	mean ± STD	% change	*P*	% change	*P*	% change	*P*
Apolipoprotein B48, mg/Ml									
0 min	5.57 ± 3.89	4.12 ± 2.25	4.58 ± 3.20	−1.45	0.105	0.46	0.492	−0.99	0.125
120 min	9.32 ± 4.82	6.32 ± 2.11	8.37 ± 4.38	−3.09	**0.049**	2.14	0.131	−0.95	0.275
Lipoprotein lipase mass, ng/ml									
0 min	41.90 ± 9.90	43.00 ± 15.13	40.30 ± 15.26	1.10	0.175	−2.7	0.646	−1.6	0.305
120 min	39.50 ± 8.58	41.00 ± 11.51	38.80 ± 12.72	1.50	0.557	−2.2	0.445	−0.7	357
HMW adiponectin, mcg/mL	3.17 ± 1.39	3.71 ± 1.46	3.85 ± 1.70	0.55	**0.037**	0.134	0.695	0.68	**0.027**
Urinary 8-OHdG, ng/mgCr									
0 min	8.62 ± 2.55	8.54 ± 2.38	8.19 ± 2.88	−0.08	0.91	−0.35	0.734	−0.43	0.557
120 min	10.30 ± 2.09	9.08 ± 2.54	9.03 ± 2.81	−1.22	**0.027**	−0.03	0.676	−1.27	0.193
hs-CRP, mg/dL									
0 min	0.10 ± 0.10	0.07 ± 0.09	0.07 ± 0.07	−0.03	**0.01**	0.0004	0.941	−0.0229	0.16
120 min	0.10 ± 0.10	0.07 ± 0.09	0.08 ± 0.07	−0.03	**0.008**	0.0049	0.461	−0.0293	0.416

[a] Triglycerides and RLP-cholesterol were log-transformed before statistical analysis. *P* values < 0.05 are shown in bold

Results are presented as mean ± SD. Differences between parameters obtained before and after anagliptin therapy were evaluated by paired Student's *t* test analysis

24 weeks except for glucose at 0 min. There were increases in CPR index and 1,5 AG and reductions in glycoalbumin, HbA1c, and HOMA-IR at 12 weeks, all of which returned to pretreatment levels at 24 weeks except for HOMA-IR. At 12 weeks, there were reductions in TC, TG, non HDL-C, and LDL-C at any time points after test meal loading, all of which returned to pretreatment levels at 24 weeks except for LDL-C. Similarly, at 12 weeks, there were reductions in RLP-C at 0 and 120 min, which returned to pretreatment levels.

At 12 weeks, there was a tendency toward reductions in postprandial apoB48 after test meal loading, which returned to pretreatment levels at 24 weeks. LPL mass did not change during the study period either in fasting or postprandial state. At 12 weeks, there were increases in serum HMW adiponectin levels and reduction in hs-CRP. And there was a significant reduction in urinary 8-OHdG at 120 min after test meal.

The present findings are consistent with previous studies that DPP-4 inhibitors such as vildagliptin and sitagliptin decrease postprandial TG, RLP-C, and apoB-48 levels after a fat-loading test in patients with type 2 diabetes [9, 10]. There are several possible mechanisms by which DPP-4 inhibition could have improved postprandial lipaemia in insulin-resistant subjects. A previous report by Zander et al. [11] showed that continuous administration of GLP-1 in patients with type 2 diabetes is associated with improved insulin sensitivity and β-cell function along with reduced FFA concentrations.

Recently, it is elucidated that GLP-1 influences intestinal TG absorption [12] potentially through gastric lipase inhibition [13], suggesting another potential mechanism underlying the beneficial impact of DPP-4 inhibitors on postprandial lipaemia. Animal studies in mice and hamsters have shown that DPP-4 inhibition or GLP-1 receptor agonists significantly reduced intestinal secretion of TG, cholesterol, and apoB-48, a finding supporting the hypothesis that GLP-1 could directly regulate lipoprotein assembly and/or secretory machinery in the enterocytes [14]. In addition, exogenous GIP infusion was associated with an enhanced chylomicron catabolism in animal models [15].

To determine potential mechanism by which TG was reduced by anagliptin treatment, we measured HMW adiponectin levels and preheparin LPL mass at 0, 12, and 24 weeks. We found HMW adiponectin levels increased at 12 weeks. This data suggest that amelioration of TG-rich lipoprotein metabolism during anagliptin treatment could be associated with increased serum adiponectin concentration. Indeed, it is reported that adiponectin levels had inverse relationships with TG levels [16]. It is also reported that circulating adiponectin is inversely correlated with postprandial area under curve (AUC) of serum triglycerides and chylomicrons [17]. A previous study shows that circulating adiponectin is associated with ApoB48, located in lipoproteins originated from the small intestine [18]. We also measured serum LPL mass at 0, 12, and 24 weeks of the study period and found no significant changes. This

may suggest that the observed changes in TG values either in fasting or postprandial states may not be due to changes in lipolysis of TG-rich lipoproteins, but instead due to decreased production of TG-rich lipoprotein from the liver or intestine. At 12 weeks, there were reductions (at 120 min after test meal) or tendency toward reductions (at 0 min after test meal) in hs-CRP, suggesting anagliptin treatment may ameliorate inflammation.

In the past decade, increasing attention has been paid to the importance of non-fasting TG as an important predictor to the development of atherosclerotic disease [19]. TG-rich lipoproteins, which consist of chylomicrons assembled by TG, dietary cholesterol, and apoB-48, are highly atherogenic and contribute to the development of coronary heart disease. Thus, the increased risk of cardiovascular events associated with non-fasting TG concentrations may reflect atherogenic properties of TG-rich lipoproteins generated during the postprandial period [2]. It is suggested that postprandial lipemia contributes to the production of pro-inflammatory cytokines and oxidative stress, resulting in endothelial dysfunction [20].

For hypoglycemic effect, we found increases in the insulinogenic index as well as reductions in HOMA-R, GA, and FPG at 12 weeks. This suggests that anagliptin treatment caused improvement in both insulin secretion and insulin resistance. Indeed, Nakaya et al. [6] reported that in mice, high-dose anagliptin treatment improved glucose tolerance by suppression of body weight gain and amelioration of insulin resistance, whereas low-dose anagliptin treatment improved glucose tolerance by enhancing insulin secretion.

The present study also suggests that oxidative stress in postprandial states may be ameliorated by anagliptin treatment.

The limitations of this study are that the sample size is small and study being done in a single arm, and the findings will need to be confirmed in placebo-controlled trials.

In conclusion, the present findings suggest that in men with type 2 diabetes, anagliptin improves hyperlipidemia in fasting and postprandial state as well as glycemic controls, contributing to the prevention of the development of atherosclerosis.

Conflict of interest There is no conflict of interest for all of the authors regarding this work.

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