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Efficacy of metformin on postprandial plasma triglyceride concentration by administration timing in patients with type 2 diabetes mellitus: A randomized cross-over pilot study

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ABSTRACT

Aims/Introduction: Preprandial metformin administration significantly reduces postprandial plasma triglyceride levels in animal studies by reducing intestinal absorption through delayed gastric emptying. However, this effect has not been shown in a clinical study. Therefore, we planned to investigate the efficacy of preprandial metformin administration on postprandial hypertriglyceridemia and the related gastrointestinal effects in patients with type 2 diabetes mellitus.

Materials and Methods: A total of 11 patients taking single-dose metformin at 500–1,000 mg, with non-fasting plasma triglyceride levels of 150–1,000 mg/dL, were recruited at a single university hospital. The difference between preprandial and postprandial metformin administration on postprandial hypertriglyceridemia was examined by a meal test. The gastrointestinal effects of metformin, including stomach heaviness, heartburn and satiety, were also assessed using a visual analog scale.

Results: The mean bodyweight of patients was 80.6 kg (body mass index 27.9 kg/m^2), and the mean non-fasting plasma triglyceride level was 275.9 ± 57.0 mg/dL. The area under the curve of triglyceride during the meal test was significantly lower in the preprandial protocol than in the postprandial protocol ($P < 0.05$). Compared with postprandial administration, preprandial administration of metformin increased satiety ($P = 0.036$) without stomach heaviness or heartburn.

Conclusions: Preprandial metformin administration significantly reduced plasma triglyceride level during meal testing without marked exacerbation of gastrointestinal adverse effects. The present results suggest that a simple change in the timing of metformin administration represents a novel approach for enhancing triglyceride-lowering strategies in patients with type 2 diabetes mellitus and postprandial hypertriglyceridemia.

INTRODUCTION

Dyslipidemia is common in patients with type 2 diabetes mellitus, affecting approximately 50% of this population[1]. The characteristic features of diabetic dyslipidemia with insulin resistance are increased levels of triglyceride (TG), including very low-density lipoprotein and chylomicron particles, reduced high-density lipoprotein cholesterol and increased low-density lipoprotein cholesterol. With regard to hypertriglyceridemia, approximately 30–40% of patients with diabetes have TG levels >200 mg/dL[2]. Recently, hypertriglyceridemia has been evaluated as a potential residual risk factor of cardiovascular disease after statin therapy. In particular, postprandial hypertriglyceridemia is strongly associated with an increased risk of cardiovascular disease[3]. Small chylomicron remnants, generated from chylomicrons in the postprandial state, are recognized as a risk factor for coronary artery disease[4,5]. In addition, postprandial hypertriglyceridemia has been shown to be independently associated

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with carotid IMT in patients with type 2 diabetes mellitus[6]. Therefore, postprandial dyslipidemia might represent a potential target for cardiovascular disease prevention.

Metformin is first-line treatment for type 2 diabetes mellitus globally, and its TG-lowering effect in the fasting and postprandial states was first reported in the 1990s[7,8]. This effect, similar to its glucose-lowering effect, was reported to be dose-dependent[9]. Recently, we found that the administration of high-dose metformin before fat loading significantly reduced postprandial plasma TG levels compared with administration after fat loading in an animal model[10]. We therefore concluded that the major mechanism underlying the TG-lowering effect of metformin is a reduction in intestinal absorption through delayed gastric emptying, and not an increase in fat oxidation in the peripheral tissues. However, no clinical studies to date have reported the effect of the timing of metformin administration.

Therefore, we examined the efficacy of metformin on postprandial hypertriglyceridemia and the gastrointestinal (GI) adverse effects related to delayed gastric emptying in Japanese patients with type 2 diabetes mellitus.

METHODS

Study design

We carried out a randomized, open-labeled, cross-over pilot study to investigate the effects of metformin administration on postprandial hypertriglyceridemia in patients with type 2 diabetes mellitus (Figure 1a,b). The primary end-point was the difference in the change in postprandial serum TG levels between preprandial (pre-Met) and postprandial (post-Met) metformin administration. Key secondary outcomes were changes in blood glucose, serum insulin and active glucagon-like peptide-1 (GLP-1) concentrations. A visual analog scale (VAS) was used to assess gastrointestinal symptoms and satiety as secondary outcomes. This study was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the ethics committee of Shiga University of Medical Science on 22 March 2016. The study is registered with UMIN (UMIN000022699), and all participants gave written informed consent to participate.

Patients

Inclusion criteria were type 2 diabetes mellitus, age <70 years and non-fasting TG levels of 150–1,000 mg/dL. All patients were hospitalized for glycemic control at the Shiga University of Medical Science Hospital, Seta-Otsu, Shiga, Japan, and all study procedures were carried out under stable glycemic control. We enrolled 11 patients who were taking metformin at a single dose of 500–1,000 mg. The main exclusion criteria were history of acute pancreatitis caused by hypertriglyceridemia, and history of slow gastric emptying, such as that caused by severe diabetic autonomic neuropathy, adhesion ileus or drugs affecting gastrointestinal motility.

Assessments

Two sets of meal tolerance tests were carried out 2–3 days apart after patients had achieved good glycemic control (fasting plasma glucose level <130 mg/dL and 2-h postprandial level <200 mg/dL). For fat loading, a meal test (cookie test) was carried out after overnight fasting for 10 h. The cookie consisted of 75 g carbohydrate (flour starch and maltose), 28.5 g fat (butter) and 8 g protein for a total of 592 kcal a carton (Saraya Corp, Osaka, Japan)[11]. At the first meal test, participants were randomized to take metformin (500–750 mg) either (1) 30 min before a test meal (pre-Met protocol) or (2) 15 min after starting a test meal (post-Met protocol). For the pre-Met protocol, a fasting blood sample (t = −30 min) was taken immediately before metformin administration. A blood sample was again taken 30 min after metformin administration (t = 0), and participants were asked to have the cookie with water within 15 min. For the post-Met protocol, a fasting blood sample (t = −30, 0 min) was taken and participants were asked to have the cookie with water, then metformin was administered 15 min after initiating cookie ingestion. For both groups, blood samples were drawn thereafter at t = 60, 120, 180 and 240 min. Plasma TG were measured at −30, 0, 60, 120, 180 and 240 min. Plasma active GLP-1 and plasma insulin were measured at t = 0 and 60 min. Blood glucose levels were measured with a glucose sensor (One Touch Ultraview®; Johnson and Johnson Co., New Brunswick, NJ, USA) at −30, 0, 60, 120, 180 and 240 min. The first and second meal tests were carried out 2–3 days apart to allow a washout period. During this period, patients maintained their first assigned treatment. At the second meal test, each participant was crossed over to the opposite protocol.

All laboratory tests were carried out at SRL Laboratories (SRL Inc., Tokyo, Japan). Plasma insulin was measured by CLIA (ARCHITECT® insulin assay; Abbott Laboratories, Abbott Park, IL, USA). Plasma active GLP-1 was measured using an enzyme-linked immunosorbent assay kit (EMD Millipore Co., St. Louis, MO, USA). Due to a technical issue, two participants had no GLP-1 measurement.

GI effects

We examined the GI effects of metformin, including stomach heaviness, heartburn and satiety, using a VAS in the pre-Met and post-Met protocols after the second meal test. VAS scores were available for 10 of the 11 patients, as one patient did not undergo the VAS assessment.

Statistical analysis

Data are presented as the mean ± standard deviation for continuous variables, and n (%) for categorical variables. Statistical analysis was carried out using appropriate parametric and non-parametric methods. Changes in continuous measures in the pre-Met and post-Met protocols were tested by paired t-test. Non-parametric methods were used for non-normally distributed values. Change in the area under the curve for 0 to

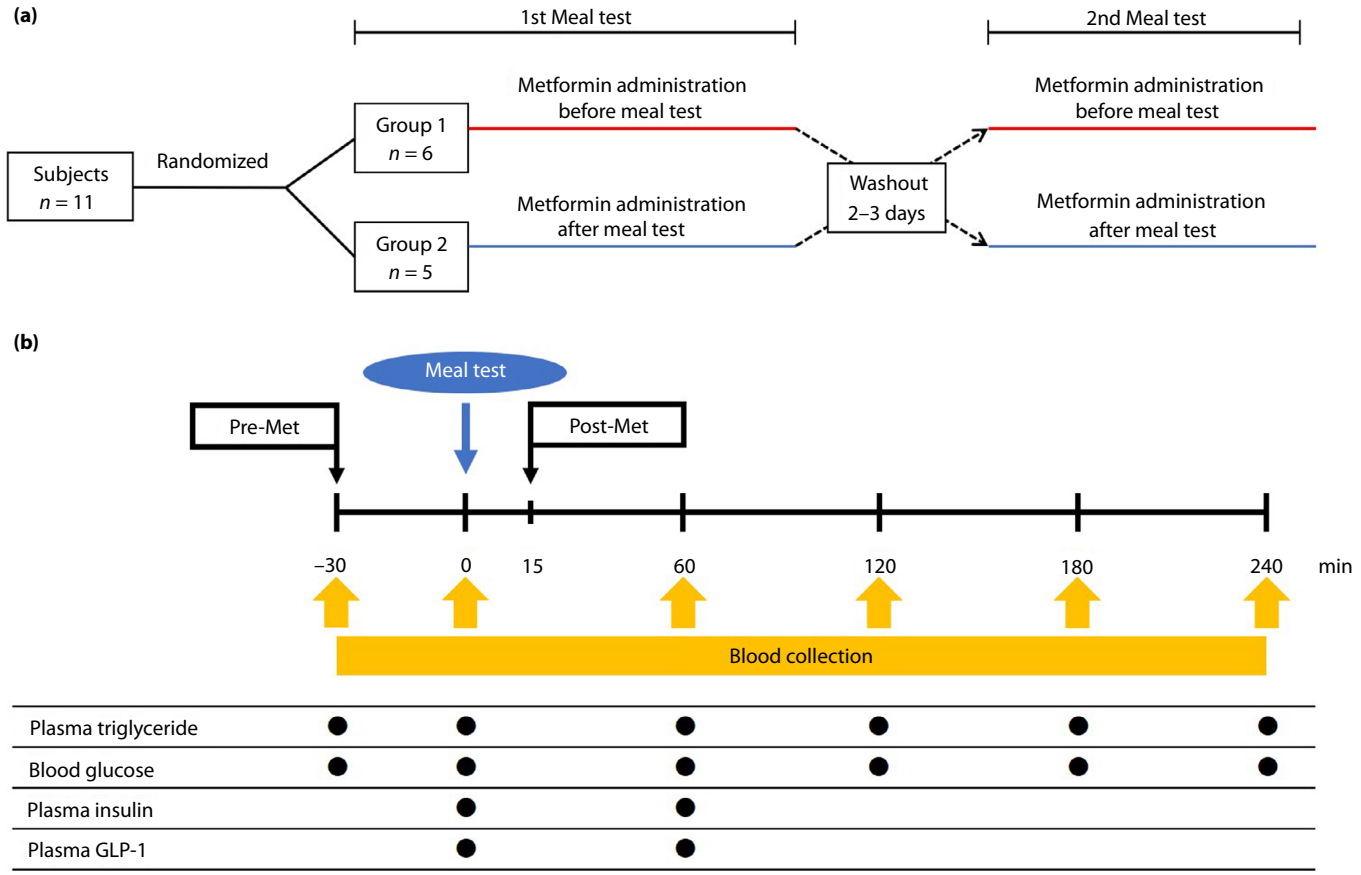

Figure 1 | (a) Study design. (b) Meal tests under the preprandial metformin administration (pre-Met; 500–1,000-mg single dose) and postprandial metformin administration (post-Met) protocols were carried out alternately. The participants ingested a cookie consisting of 75 g carbohydrate (flour starch and maltose) and 28.5 g fat. Blood was collected at fasting (–30, 0), 60, 120, 180 and 240 min after ingestion of the cookie. GLP-1, glucagon-like peptide-1.

4 h (AUC 0–4 h) for TG, AUC 0–4 h for glucose, and VAS scores for the pre-Met and post-Met protocols were analyzed using the Wilcoxon signed-rank test. To assess the relationship between change in AUC 0–1 h for active plasma GLP-1 and change in AUC 0–4 h for TG, Pearson's correlation coefficient was calculated. All analyses were carried out using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA). A P-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

The baseline characteristics of all patients immediately before hospitalization are shown in Table 1. Nine men and two women (aged 53.5 ± 12.9 years) were recruited. Mean body-weight was 80.6 kg, and mean BMI was 27.9 kg/m^2. Non-fasting plasma TG level was 275.9 ± 189.0 mg/dL, and glycated hemoglobin level was 10.2 ± 1.9%. Antidiabetic and antilipidemic medications are also shown in Table 1. Three out of 11 patients were treated with metformin 500-mg single dose, and eight out of 11 with metformin 750-mg single dose. No patient was receiving long-acting metformin. Concomitant antidiabetic drugs excluding metformin were a sodium–glucose cotransporter 2 inhibitor (n = 6), dipeptidyl peptidase-4 inhibitor (n = 2) and insulin therapy (n = 2). Six patients (54.5%) were treated with statins.

Metabolic parameters during meal testing under pre-Met and post-Met administration

The differential effects of metformin pre-Met and post-Met administration on postprandial hypertriglyceridemia were examined by meal testing with cookies. The TG, glucose and insulin results are shown in Figure 2. Compared with post-Met administration, pre-Met administration resulted in slightly lower postprandial TG levels (Figure 2a). The AUC 0–4 h for TG in the pre-Met protocol was significantly lower than that of the post-Met protocol (P = 0.032; Figure 2d). Blood glucose levels tended to be lower at 0–180 min, but were significantly higher at 240 min (P = 0.048) in the pre-Met group (Figure 2b). The AUC 0–4 h for glucose showed no difference between the two groups (Figure 2e). The changes in plasma

Table 1 | Patient characteristics at baseline

Patient characteristics	Study population (n = 11)
Male/female (n)	9/2
Age (years)	53.5 ± 12.9
Weight (kg)	80.6 ± 13.5
Body mass index (kg/m^2)	27.9 ± 4.4
HbA1c (%)	10.2 ± 1.9
Non-fasting plasma triglyceride (mg/dL)	275.9 ± 189.0
Medication	
Metformin single dose, 500/750/1,000 mg (n)	3/8/0
Glucose-lowering agents, excluding metformin	
SGLT2 inhibitor, n (%)	6 (54.5)
DPP-4 inhibitor, n (%)	2 (18.2)
Insulin therapy, n (%)	2 (18.2)
Lipid-lowering agents	
Statin, n (%)	6 (54.5)

Values are expressed as the mean ± standard deviation for continuous variables or number of patients (%). DPP-4, dipeptidyl peptidase-4; HbA1c, glycated hemoglobin; SGLT2, sodium–glucose cotransporter

insulin levels at t = 0–60 min showed no difference between the two groups (Figure 2c). The relationship between ΔAUC 0–4 h for TG (pre-Met protocol minus post-Met protocol) and ΔAUC 0–4 h for glucose showed a significant positive correlation (r = 0.78, P < 0.005; Figure 2f).

GI effects of metformin pre-administration using VAS

Pre-Met administration increased satiety compared with post-Met administration (P = 0.036; Figure 3a). In contrast, negative GI symptoms, such as stomach heaviness and heartburn, were unchanged (Figure 3b,c).

Effects of plasma active GLP-1 on postprandial TG concentrations

It is well established that GLP-1 slows gastric emptying. Plasma active GLP-1 levels at 0 min and 60 min were measured in both the pre-Met and post-Met protocols, because several reports have shown that metformin increases plasma active GLP-1 levels. However, there were no differences in mean GLP-1 level observed between the two groups (Figure 4a).

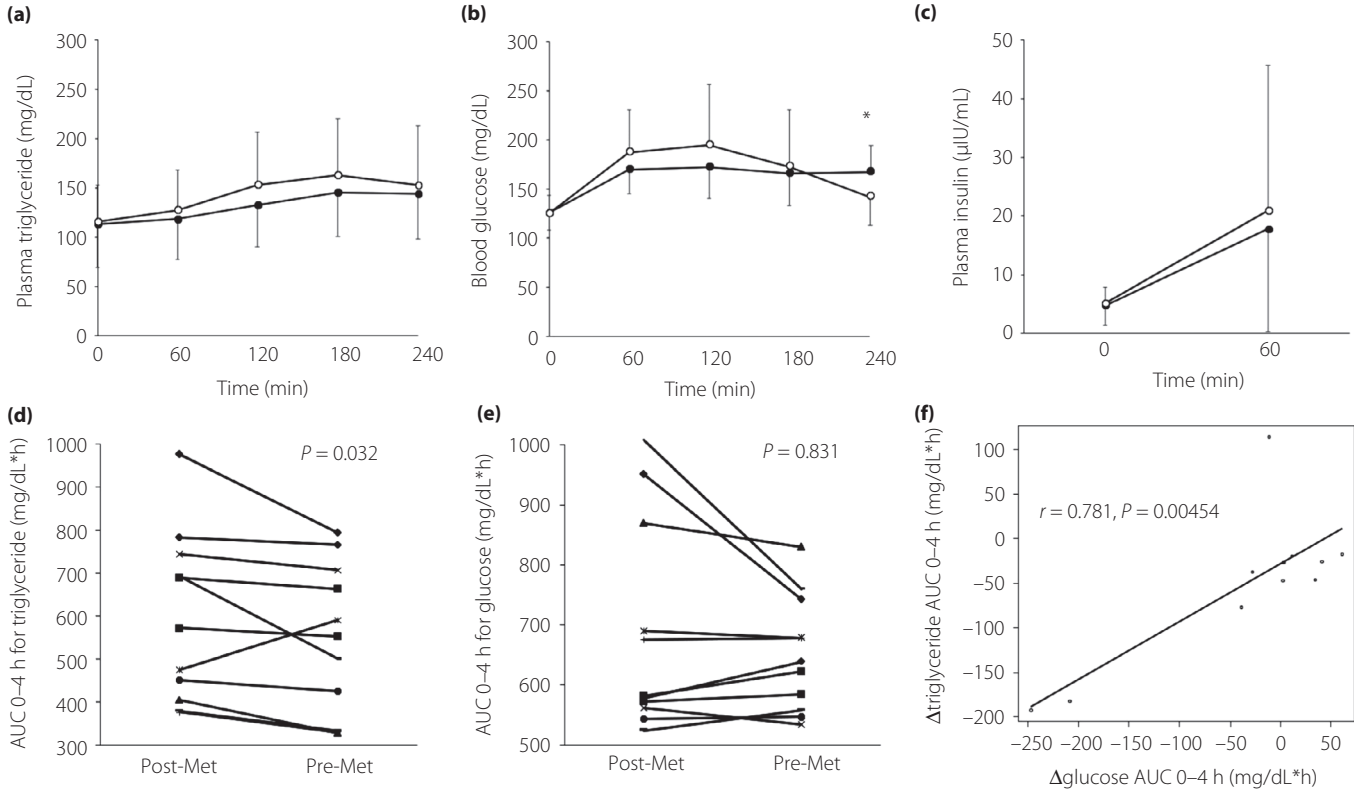

Figure 2 | Effect of the preprandial metformin administration (pre-Met) protocol on efficacy parameters in 11 patients with type 2 diabetes mellitus. Change in (a) plasma triglyceride (TG) levels, (b) blood glucose levels and (c) plasma insulin levels during the meal test. Data are the mean ± standard deviation. *P < 0.05 versus postprandial metformin administration (post-Met) protocol by paired t-test. Filled circle: pre-Met protocol; open circle: post-Met protocol. (d) Change in TG area under the curve for 0 to 4 h (AUC 0–4 h) under the pre-Met and post-Met protocols. (e) Change in glucose AUC 0–4 h under the pre-Met and post-Met protocols. Change in TG AUC 0–4 h and glucose AUC 0–4 h were analyzed using the Wilcoxon signed-rank test. (f) Correlation between ΔTG AUC 0–4 h (pre-Met protocol minus post-Met protocol) and Δglucose AUC 0–4 h. The relationship between ΔTG AUC 0–4 h and Δglucose AUC 0–4 h was analyzed using Pearson's correlation coefficients.

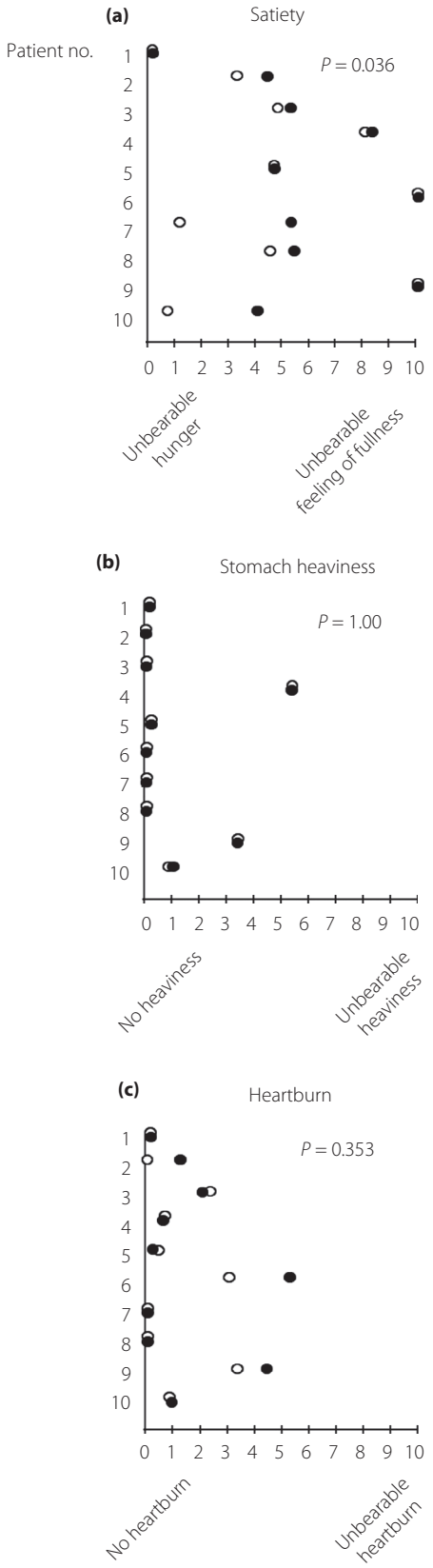

Figure 3 | Change in visual analog scale scores for gastrointestinal adverse effects of metformin. (a) Satiety, (b) stomach heaviness and (c) heartburn. Change in visual analog scale scores in preprandial metformin administration (pre-Met) and postprandial metformin administration (post-Met) protocols were analyzed using the Wilcoxon signed-rank test. Filled circle: pre-Met protocol; open circle: post-Met protocol.

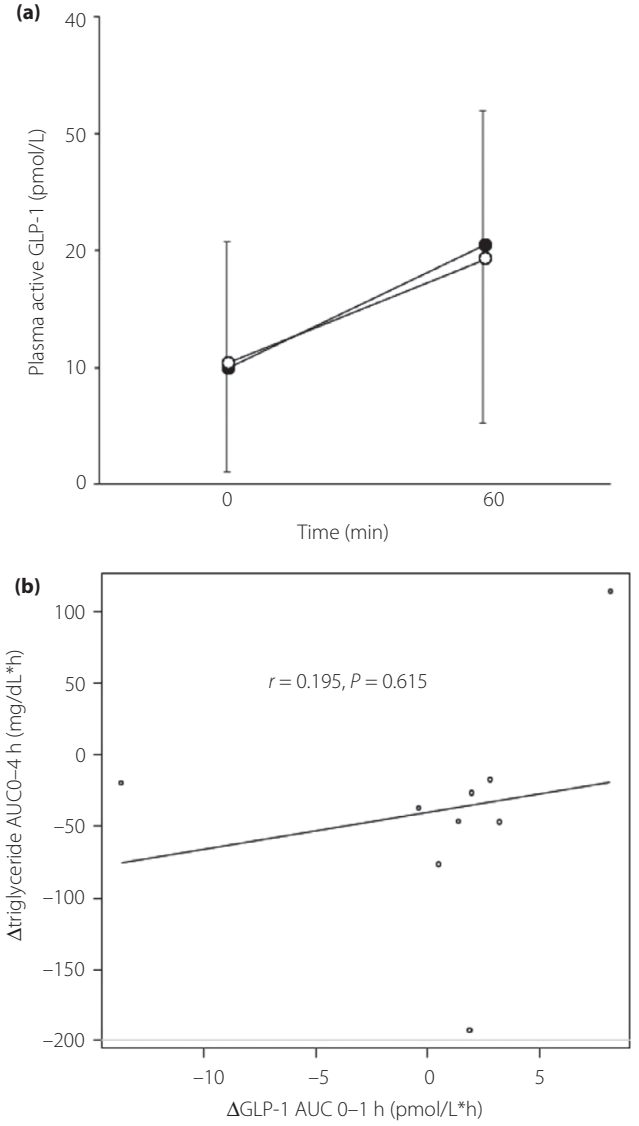

Figure 4 | Correlation between the change in plasma-active glucagon-like peptide-1 (GLP-1) and triglyceride (TG) area under the curve for 0 to 4 h (AUC 0–4 h). (a) Change in plasma active GLP-1 levels during the meal test. Data are the mean ± standard deviation. Filled circle: pre-Met protocol; open circle: post-Met protocol. (b) Correlation between ΔGLP-1 AUC 0–1 h (pre-Met protocol minus post-Met protocol) and ΔTG AUC 0–4 h. The relationship between ΔGLP-1 AUC 0–1 h and ΔTG AUC 0–4 h was analyzed using Pearson's correlation coefficients.

Furthermore, no association was observed between ΔAUC 0–1 h for GLP-1 (pre-Met protocol minus post-Met protocol) and ΔAUC 0–4 h for GLP-1 (Figure 4b).

DISCUSSION

The present study showed two major findings. First, pre-Met administration significantly reduced postprandial TG levels compared with post-Met administration. Second, pre-Met administration increased satiety without marked exacerbation of adverse effects, such as stomach heaviness and heartburn.

Pre-Met administration significantly reduced postprandial TG compared with post-Met administration in this clinical study. In particular, participants with a larger reduction in AUC 0–4 h for glucose showed larger effects on AUC 0–4 h for TG. These findings are in agreement with those of our previous pre-clinical study in which pre-Met administration lowered plasma TG and glucose levels in mice[10]. Furthermore, the present findings are consistent with a previous clinical study that showed lower glucose concentrations with pre-Met administration before oral glucose tolerance testing[12]. However, unlike the previous clinical study, we found that pre-Met administration increased plasma glucose levels at $t = 240$ min with a delayed peak time compared with post-Met administration. A possible reason for this late increase in blood glucose levels with a peak delay might be delayed gastric emptying, which we previously reported as a principle mechanism of the TG-lowering effect of metformin in mice[10], although the mechanism remains unknown. Several studies have reported that GLP-1 analogs delay gastric emptying and significantly lower postprandial TG levels in patients with type 2 diabetes mellitus [13–16]. Recently, metformin has been shown to increase plasma GLP-1 levels[17–19]. These reports indicate that GLP-1 might be a key factor in lowering postprandial TG levels with pre-Met administration through delayed gastric emptying. In the present study, no difference in plasma GLP-1 levels was observed between pre- and post-Met administration. However, plasma GLP-1 might have entered the capillaries and been rapidly degraded by dipeptidyl peptidase IV[20]. It has also been reported that GLP-1 released from L cells can immediately activate GLP-1 receptors located on vagal sensory neurons in the portal vein[21]. Furthermore, a recent report showed that stimulation of the vagal afferent nerve by portal-vein GLP-1 can delay gastric emptying[22]. Therefore, further evaluation of the portal vein GLP-1 levels and postprandial TG-lowering effects of metformin might be of interest.

GI adverse effects, such as heartburn, nausea, stomach heaviness and diarrhea, are the main complaints of patients receiving metformin and might reduce medication adherence. Analysis of the VAS data showed that pre-Met administration increased satiety compared with post-Met administration. Thus, there is a possibility that pre-Met administration for a prolonged period can improve TG and glucose metabolism by suppression of appetite and subsequent weight loss. Although not statistically significant, in contrast, pre-Met administration increased the sensation of heartburn in some patients. Attention should be paid to the exacerbation of adverse GI effects when changing from post-Met administration to pre-Met administration.

Several limitations of the present study should be noted. First, participants took metformin (500–750-mg single dose) 30 min before a meal in the pre-Met protocol. However, the optimal timing and dose of metformin remain unclear. Second, we could not evaluate the peak of postprandial plasma TG levels in a short time of 4 h. It will be necessary to evaluate the effects of delayed gastric emptying on TG levels at later time points. Third, a single dose of metformin was compared between the pre-Met and post-Met protocols. Thus, the long-term effects of pre-Met administration remain unknown. Fourth, the present study was carried out in patients who had achieved good glycemic control. Thus, the effect of pre-Met administration for patients with insufficient glycemic control requires further clinical investigation. Finally, the study population was small, and a larger confirmative study is therefore required.

In conclusion, this is the first study to show that metformin administration 30 min before a meal significantly reduced postprandial plasma TG levels without marked exacerbation of gastrointestinal adverse effects. The present results suggest that a simple change in the timing of metformin administration represents a novel approach for enhancing TG-lowering strategies in patients with type 2 diabetes mellitus and postprandial hypertriglyceridemia.

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DISCLOSURE

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