Coingestion of Acylglycerols Differentially Affects Glucose-Induced Insulin Secretion via Glucose-Dependent Insulinotropic Polypeptide in C57BL/6J Mice

Akira Shimotoyodome, Daisuke Fukuoka, Junko Suzuki, Yoshie Fujii, Tomohito Mizuno, Shinichi Meguro, Ichiro Tokimitsu, and Tadashi Hase

Biological Science Laboratories (A.S., D.F., J.S.,Y.F., S.M., T.H.) and Health Care Food Research Laboratories (T.M., I.T.), Kao Corporation, Tochigi 321-3497, Japan

The precise role of fat in postprandial glycemia and insulinemia has not been thoroughly researched because postprandial blood glucose and concurrent insulin secretion are largely assumed to be proportional to carbohydrate intake. Recent studies have suggested that dietary fat differentially regulates the postprandial insulin response. To explore this, we examined the effects of coadministered fat on glucose-induced glycemia and insulinemia in C57BL/6J mice. The insulin response to glucose was augmented by the addition of glycerol trioleate (TO) in a dose-dependent manner, which was associated with enhanced glucose transport from the circulation to muscle and adipose tissues. To investigate the mechanism underlying fat-induced hyperinsulinemia, we examined the release of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1. TO increased GIP secretion, whereas glucagon-like peptide-1 secretion was unaffected. TO-induced hyperinsulinemia was significantly attenuated by the pretreatment of mice with a specific GIP antagonist. Diacylglycerol (DAG) promoted lower postprandial GIP and triglyceride responses and, when ingested with glucose, a lower insulin response compared with triacylglycerol of a similar fatty acid composition. Pluronic L-81, an inhibitor of chylomicron formation, reduced not only the triglyceride response but also TO-induced GIP secretion, indicating that the lower GIP response after DAG ingestion may be associated with retarded chylomicron formation in the small intestine. We conclude that dietary fat augments glucose-induced insulinemia via gut-derived GIP and, thereby, influences postprandial nutrient metabolism in mice. DAG promotes a lower GIP and thereby reduced insulin responses compared with triacylglycerol, which may differentially influence postprandial energy homeostasis. **(*Endocrinology* 150: 2118–2126, 2009)**

Postprandial hyperglycemia has recently been recognized as an important risk factor for cardiovascular disease not only among persons with diabetes but also among the general population (1). Recent studies have shown that high-level postprandial glucose and insulin responses, resulting from carbohydrate-rich diets, are associated with undesirable lipid profiles (2), increased body fat (3, 4), and the development of insulin resistance in rodents (5) and humans (6). Prolonged or high levels of postprandial insulinemia are also thought to contribute to the development of insulin resistance and associated diseases (7–14). Although the standardized measurement of the glycemic index and insulinemic response to the diet is of both theoretical and practical significance, the clinical relevance of the glycemic index remains a subject of debate, mostly because the glycemic response to individual foods is not maintained in mixed meals due to the confounding effects of dietary factors other than carbohydrate, *i.e.* protein and fat (15–19).

The precise role of fat and protein in postprandial glycemia and insulinemia has not been thoroughly investigated because postprandial blood glucose and concurrent insulin secretion are

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Abbreviations: ANTGIP, GIP receptor antagonist; AUC, area under the curve; BW, body weight; DAG, diacylglycerol; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; K_G, glucose elimination constant; PL, Pluronic L-81; PLSD, protected least significant difference; TAG, triacylglycerol; TG, triglycerides; TO, glyceryl trioleate.

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largely assumed to be proportional to carbohydrate intake (20). One of the aims of this study was to clarify how the co-ingestion of fat affects the increase in blood glucose and insulin levels after carbohydrate intake. We examined the effects of coadministered fat on glucose-induced glycemia and insulinemia in C57BL/6J mice. Because intragastrically administered fat increased the postprandial insulin response, we examined gut-derived incretins, the two most important being glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (21). We herein report that fat ingestion potentiates glucose-induced insulinemia via gut-derived GIP and, thereby, results in the increased incorporation of dietary nutrients into muscle and adipose tissues.

Prior studies in animals and humans have shown that dietary diacylglycerol (DAG) oil leads to the suppression of body fat accumulation, body weight loss, improved glucose tolerance, and lower postprandial lipemia compared with triacylglycerol (TAG), of a similar fatty acid composition (22–25). The structural and metabolic characteristics of DAG compared with TAG are believed to be responsible for these beneficial effects.

More recently, studies have shown that the ingestion of a DAG oil-rich diet results in a lower postprandial insulin response compared with TAG oil in humans (26, 27). These findings suggest a differential regulation of the postprandial insulin response and a possible mechanism for a postprandial increase in energy expenditure mediated by DAG. However, the underlying mechanisms for this action still remain to be elucidated.

For the above reasons, this study was also designed to clarify the underlying mechanism of DAG action which lowers the postprandial insulin response. We investigated the effect of DAG on blood glucose, insulin, and GIP. We also report that DAG ingestion results in lower GIP and insulin responses, and that this may be related to the characteristic metabolism of DAG in the small intestine and a possible mechanism explaining the anti-obesity effect of DAG.

Materials and Methods

Materials

All reagents for experiments, as well as for analytical procedures, were from Sigma-Aldrich Japan K.K. (Tokyo, Japan) unless otherwise stated.

Animals

Male C57BL/6J mice (7–8 wk old; CLEA Japan, Inc., Tokyo, Japan) were housed five per cage in a temperature- and a relative humidity-controlled (23 ± 2.0 C, 55 ± 10%) room with a 12-h light, 12-h dark cycle with lights on at 0700 h. Mice were fed a standard chow consisting of 3.47 kcal/g, with 4.6% fat, 51.4% carbohydrate, and 24.9% protein (CE-2; CLEA Japan). Food and water were provided *ad libitum*. All animals were housed for 1–2 wk before use. All animal experiments were conducted in the Experimental Animal Facility of Kao Tochigi Institute. The Animal Care Committee of Kao Tochigi Institute approved the present study. All experiments strictly followed the guidelines of that committee.

Oral glucose and fat load study

Overnight-fasted mice were anesthetized through the inhalation of diethyl ether, and administered D-(+)-glucose [2 mg/g body weight (BW)] through a gastric tube with or without the addition of glyceryl trioleate (TO, 0.5–2 mg/g BW). Lecithin (from egg yolk, 0.08 mg/g BW; Kanto Chemical Co., Inc., Tokyo, Japan) was included in all test solutions containing oil, and these were subsequently sonicated three times for 60 sec with a 1-min interval of cooling on ice to obtain stable emulsions (Sonifier 450; Branson Ultrasonics Co., Danbury, CT). The administration volume was adjusted to 40 μl/g BW. We preliminarily confirmed that the minimal amount of lecithin did not affect blood glucose and insulin responses after gastric glucose gavage. Accordingly, we used the glucose solution as a control sample. Blood samples (~50 μl) were collected from the orbital sinus under anesthesia with diethyl ether inhalation immediately before and at indicated times after gastric gavage using a heparinized capillary tube (75 mm length; Drummond scientific Co., Broomall, PA). Blood samples were kept on ice until plasma preparation. After centrifugation at 11,000 rpm for 5 min (Micro Hematocrit Centrifuge model 3200; Kubota Co., Tokyo, Japan), plasma was stored at −80 C until analysis.

For GLP-1 analysis, we collected whole blood samples via the abdominal vein for single blood time points (0, 10, 30, or 60 min) because 200-μl plasma samples were necessary to determine active GLP-1. Thirty-five overnight-fasted mice were divided into seven groups. One group was designated the 0-min group, and blood samples (~400 μl) were taken from the abdominal vein under anesthesia with diethyl ether inhalation immediately before gastric gavage. Three groups were administered glucose alone (2 mg/g BW), and the remaining three were administered glucose plus TO (2 mg/g BW) in the same manner as described above. Blood samples were taken from the glucose- and glucose plus TO-administered groups (one group each) at 10, 30, and 60 min after gastric gavage via the abdominal vein under anesthesia. Blood samples were collected into capillary blood collection tubes (CAPIJECT with EDTA-2Na; Terumo Medical Co., Tokyo, Japan) containing dipeptidyl peptidase IV inhibitor (Millipore, Tokyo, Japan) and maintained on ice until plasma preparation. After centrifugation, plasma was stored at −80 C until GLP-1 analysis.

Tissue distribution of radiolabeled glucose

Overnight-fasted mice were administered glucose (2 mg/g BW) containing D-[U-^{14}C]glucose (4 nCi/g BW; GE Healthcare Bio-Sciences KK, Tokyo, Japan) with (2 mg/g BW) or without TO. Mice were placed in individual, airtight metabolic cages (Metabolica type MM-CO_2; Sugiyama-gen, Tokyo, Japan) and maintained for 2 h at 22 ± 2 C. Air was drawn through the cages at 150 ml/min. The air entering the cage was first passed through 5 N sodium hydroxide solution to remove atmospheric carbon dioxide and then passed through silica gel blue (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to remove water vapor. The gas expired by the mice was trapped in a trough gas collection chamber containing 5 N sodium hydroxide solution (40 ml). A 2-ml aliquot of the expired $^{14}CO_2$ was collected at 10, 30, 60, and 120 min after administration of the tracer. Blood time points were also collected at the same time in the same manner as described above. The mice were euthanized by collecting whole blood (~ 500 μl) from the abdominal vein under anesthesia with diethyl ether inhalation; then, the liver, gastrocnemius muscle, and epididymal fat were collected for radioactivity measurement.

GIP receptor antagonist (ANTGIP)

Synthetic peptide corresponding to mouse ANTGIP [7-30GIP(NH_2)], a GIP fragment identified as a specific ANTGIP (28), was obtained from the Oriental Yeast Co., Ltd. (Tokyo, Japan). We preliminarily confirmed that ANTGIP reduced GIP-promoted insulin secretion from isolated mouse pancreatic islets.

Overnight-fasted mice were sc administered saline or ANTGIP (100 pmol/g BW). After 30 min, either saline- or ANTGIP-administered mice were administered glucose with or without TO through a gastric tube, and blood samples were collected in the same manner as described above.

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Oral administration of DAG oil

DAG or TAG oil was prepared by the esterification of oleic acid (purity >85%, NOF Co., Tokyo, Japan) with glycerol in the presence of immobilized lipase (29, 30) and purified further with open column liquid chromatography (31). The ester distributions of acylglycerols and the fatty acid compositions of TAG and DAG (by weight) were determined by gas chromatography. The fatty acid composition of the DAG oil was very similar to that of the TAG oil (oleic/stearic/linoleic acid = 85.2/3.3/6.9% *vs.* 84.9/3.4/5.8%, DAG *vs.* TAG). The DAG concentration of the DAG oil was 94.2/100 g, and the ratio of 1(3),2- to 1,3-DAG was 30.0:64.2.

Albumin (from bovine serum, 0.04 mg/g BW) was included in addition to lecithin (0.04 mg/g BW) in all test solutions containing oil, and these were subsequently sonicated in the same manner as described above. The addition of a minimal amount of albumin was necessary to obtain stable emulsions with DAG oil. After being determined by laser light-scattering spectrometry (SALD-2100; Shimadzu, Kyoto, Japan), the particle size distributions of the two emulsions were found to be identical, with mean diameters of 1.45 ± 0.25 μm in TAG and 1.23 ± 0.21 μm in DAG oil. We used the glucose solution as a control sample because the minimal amount of lecithin and albumin did not affect blood glucose and insulin responses after gastric glucose gavage in a preliminary experiment.

Overnight-fasted mice were administered either TAG or DAG oil (2 mg/g BW) alone or together with glucose (2 mg/g BW), and blood time points were collected in the same manner as described above. Blood samples were kept on ice until plasma preparation. After centrifugation, plasma was stored at −80 C until analysis.

Effect of Pluronic L-81 on fat-induced GIP secretion

Overnight-fasted mice were administered TO (2 mg/g BW) emulsified using lecithin with or without Pluronic L-81 (PL, 0.31 mg/g BW; BASF, Tokyo, Japan), and blood samples were collected in the same manner as described above. Blood samples were kept on ice until plasma preparation. After centrifugation, plasma was stored at −80 C until analysis.

Plasma analysis

Blood glucose was determined by a blood glucose self-monitoring device (Accu-Chek Comfort; Roche Diagnostics Co., Tokyo, Japan) immediately after blood collection. Plasma insulin was determined using a rat/mouse insulin ELISA kit and rat insulin as standard (Morinaga Institute of Biological Science, Inc., Yokohama, Japan). Triglycerides (TG) were assessed with the Triglyceride E-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Total GIP and active GLP-1 were measured using an ELISA kit for total GIP and a GLP-1 active ELISA kit (Linco Research Inc., St. Charles, MO), respectively.

Statistical analysis

Values are means ± SEM. Glycemic, insulinemic, and GIP responses were assessed by calculating the incremental area under the curve (AUC) using the trapezoid rule from 0–120 min for plasma glucose, from 0–30 min for insulin, and from 0–60 min for GIP. The glucose elimination constant (K_G) was estimated as the glucose elimination rate between 10 and 30 min: K_G (percent per minute) = ([glucose]10 min − [glucose]30 min)/[glucose]10 min/20 min.

Pearson's correlation coefficients were obtained to estimate the linear correlation between glucose and insulin levels. Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were used when comparing values between the groups. When more than two groups were compared, statistical analysis was conducted using a one-way ANOVA and subsequently employing Fisher's protected least significant difference (PLSD) multiple comparison (STATVIEW for Windows version 5.0; SAS Institute Inc., Cary, NC).

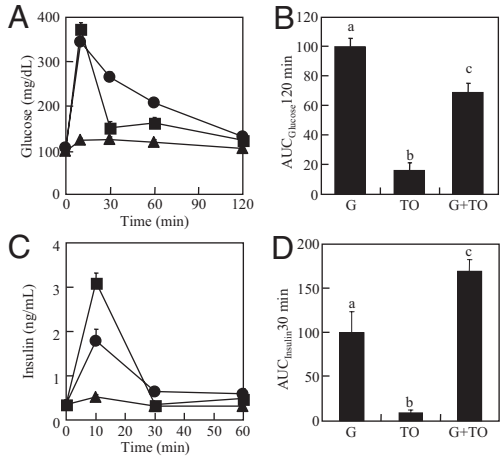

FIG. 1. Plasma concentrations of glucose (A) and insulin (C) and AUC immediately before and 10, 30, 60, and 120 min after the administration of glucose alone (2 mg/g BW, ●, G), triolein alone (2 mg/g BW, ▲, TO), or both together with glucose (2 mg/g BW each, ■, G+TO) through gastric gavage in overnight-fasted anesthetized male C57BL/6J mice. AUC are calculated from 0–120 min for glucose (B) and from 0–30 min for insulin (D). Data are expressed as means ± SEM; n = 6 in each group. Statistical analysis of the AUC was conducted using a one-way ANOVA and subsequently Fisher's PLSD multiple comparison. a–c, Means not sharing a given *letter* differ significantly ($P < 0.05$).

Results

Glucose and insulin responses

Glucose administration increased plasma glucose levels at 10 min, and thereafter, the levels declined (Fig. 1A). Insulin levels peaked at 10 min after glucose administration and thereafter declined, reaching the baseline after 30 min (Fig. 1C). After fat (TO) administration, plasma levels of glucose and insulin remained stable throughout the study period (Fig. 1, A and C). When TO was administered together with glucose, the insulin level was significantly increased at 10 min, followed by a marked decline of blood glucose at 30 min (Fig. 1, A and C). The peak TG level (at 60 min) was also decreased after the administration of TO with glucose compared with TO alone (182.2 ± 21.9 *vs.* 235.4 ± 10.6 mg/dl, respectively). Both the glucose and TG responses were significantly decreased after glucose plus TO compared with glucose (~30% decrease in $AUC_{Glucose}$120 min, Fig. 1B) or TO alone (>40% decrease in AUC_{TG}120 min).

The coadministration of TO with glucose increased peak insulin levels and decreased blood glucose at 30 min in a dose-dependent manner (Fig. 2, C and A, respectively). The coadministration of TO with glucose did not affect peak blood glucose levels. Blood glucose at 30 min was negatively ($r = -0.782$; $P < 0.001$; Fig. 2E) and K_G (the glucose elimination rate between 10 and 30 min) was positively ($r = 0.675$; $P < 0.001$; Fig. 2F) associated with the peak insulin level at 10 min. The glucose-induced insulin response (AUC) for 30 min was augmented dose-dependently upon TO administration (~2-fold increase at maximum; $P < 0.01$; Fig. 2D), which was associated with a decreased glucose response (AUC) for 120 min (~30% decrease at the maximum; $P < 0.001$) (Fig. 2B).

Tissue distribution of orally administered glucose

Table 1 shows the tissue distribution of radioactivity derived from orally administered glucose after 120 min. Glucose-derived

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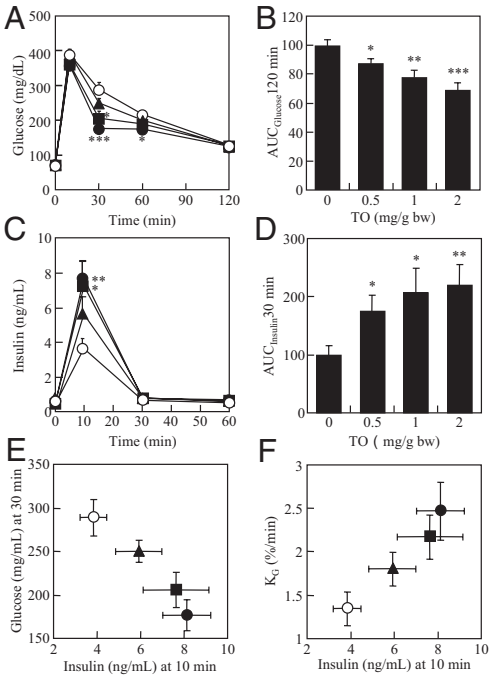

FIG. 2. Plasma concentrations of glucose (A), insulin (C), and the relationship between plasma insulin and plasma glucose (E) or K_G (F) after the administration of glucose alone (○, 2 mg/g BW, G) or together with 0.5 (▲), 1 (■), or 2 (●) mg/g BW of TO in fasted anesthetized male C57BL/6J mice. AUC are calculated from 0–120 min for glucose (B) and from 0–30 min for insulin (D). Data are expressed as means ± SEM; n = 10 in each group. Pearson's correlation coefficients were obtained to estimate the linear correlation between glucose and insulin levels. Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were used when comparing values between the glucose-only group and the experimental group. *Asterisks* indicate the probability level of random differences between the groups: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

radioactivity in plasma peaked at 10 min after glucose administration and thereafter declined. When TO was administered together with glucose, the radioactivity of plasma was significantly lower at 30 min compared with glucose alone. Glucose-derived radioactivity in plasma during 120 min (AUC) was significantly decreased by the addition of TO (~15%, $P < 0.05$). The addition of TO also reduced the glucose-derived radioactivity in the liver by approximately 25% ($P < 0.05$), whereas it increased both in the gastrocnemius muscle (~27%, $P < 0.01$) and white adipose tissue (~33%, $P < 0.05$). Expired radioactivity was unaffected by the addition of TO.

Incretin responses

Figure 3 shows the plasma concentrations of total GIP (Fig. 3A) and active GLP-1 (Fig. 3C) after the administration of glucose alone or glucose together with TO. Both oral glucose and TO increased plasma GIP levels at 10 min, and thereafter, GIP levels declined, reaching the baseline after 60 min. The plasma GIP response for 60 min tended to be higher after TO than glucose administration (~2-fold; $P = 0.053$ for AUC_{GIP}60 min; Fig. 3B). When TO was added to glucose, the GIP level was significantly increased at either 10 or 30 min compared with glucose alone ($P < 0.01$). The plasma GIP response for 60 min (AUC_{GIP}60 min) was additively increased when glucose and TO were administered together. The active GLP-1 concentration tended to be increased after 30 min by the addition of TO, but this was not significant (Fig. 3C).

Effect of ANTGIP on fat-induced increase in insulin secretion

Figure 3, D and E, shows the effect of ANTGIP on blood glucose and insulin responses after the administration of glucose with or without TO. Blood glucose and insulin responses after glucose administration did not differ between saline- and ANTGIP-pretreated mice. In contrast, the insulin response after gastric gavage with glucose plus TO was significantly lower in ANTGIP-pretreated than in saline-treated mice ($P < 0.05$). Peak insulin levels of saline-pretreated mice were significantly higher in the glucose- plus TO-administered group compared with the glucose-administered group. In contrast, peak insulin levels of ANTGIP-pretreated mice were similar between the glucose- and the glucose- plus TO-administered groups. Blood glucose levels at 30 min in saline-pretreated mice were significantly lower in the glucose- plus TO-administered group compared with the glucose-administered group ($P < 0.05$). In contrast, those of ANTGIP- pretreated mice were similar between glucose- and glucose- plus TO-administered groups.

TABLE 1. Tissue distribution of orally administered [^{14}C]glucose after the administration of glucose (G) alone or together with TO through gastric gavage in anesthetized C57BL/6J mice

Tissues	Time (min)	Radioactivity ([^{14}C] DPM/g sample)		Relative radioactivity (%)		*P*
		G	G+TO	G	G+TO	
Plasma	10	13,170 ± 509	12,680 ± 252	100.0 ± 3.9	96.3 ± 1.9	NS
	30	11,738 ± 779	7,187 ± 449	100.0 ± 6.6	61.2 ± 3.8	<0.01
	60	7,638 ± 685	6,885 ± 483	100.0 ± 9.0	90.1 ± 6.3	NS
	120	3,210 ± 291	3,722 ± 227	100.0 ± 9.1	116.0 ± 7.1	NS
Plasma AUC 120 min (×10^3)		931 ± 50	791 ± 28	100.0 ± 5.3	85.0 ± 3.0	<0.05
Liver	120	48,479 ± 4,166	36,178 ± 3,218	100.0 ± 8.6	74.6 ± 6.6	<0.05
Gastrocnemius muscle	120	1,173 ± 52	1,491 ± 18	100.0 ± 4.4	127.1 ± 1.5	<0.01
Epididymal fat	120	737 ± 18	984 ± 71	100.0 ± 2.4	133.4 ± 9.7	<0.05
Expired CO_2	120	1,633 ± 6	1,712 ± 198	100.0 ± 0.4	104.9 ± 12.2	NS

Results are means ± SEM (n = 4). Unpaired Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were conducted between G and G+TO groups. DPM, Disintegrations per minute; NS, not significant.

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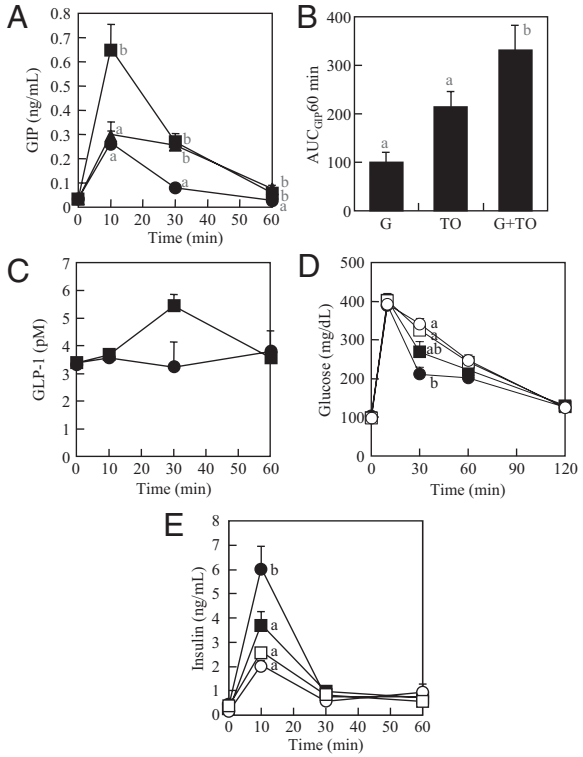

FIG. 3. Plasma concentrations of total GIP (A) and active GLP-1 (C) after the administration of glucose alone (●, G), triolein alone (▲, TO), or both together (■, G+TO) in overnight fasted anesthetized male C57BL/6J mice. AUC are calculated from 0–60 min for GIP (B). Plasma concentrations of glucose (D) and insulin (E) after the administration of glucose alone (○ and □) or TO together with glucose (● and ■) in C57BL/6J mice previously administered saline (○ and ●) or ANTGIP (100 pmol/g BW, □ and ■). Data are expressed as means ± SEM; n = 5–7 in each group. Statistical analysis was conducted using a one-way ANOVA and subsequently Fisher's PLSD multiple comparison. a and b, Means not sharing a given *letter* differ significantly ($P < 0.05$).

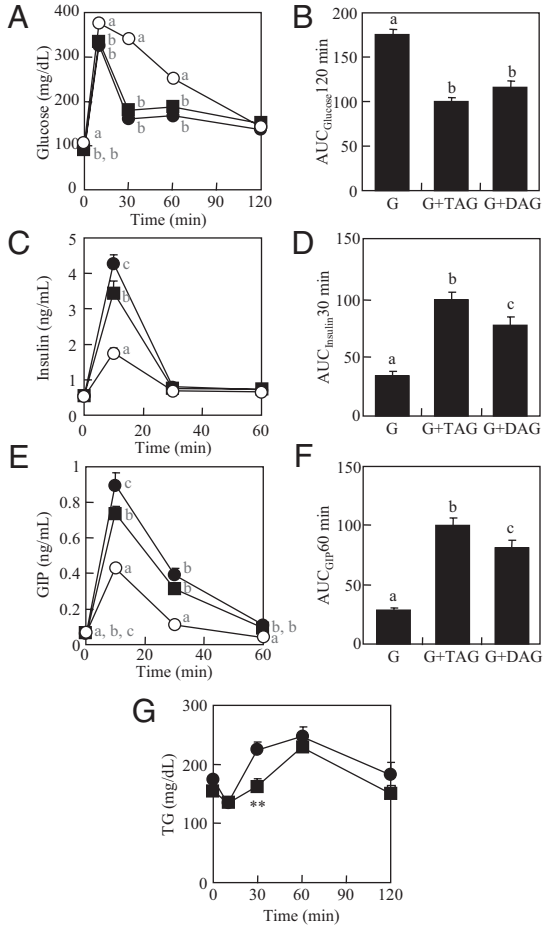

FIG. 4. Plasma concentrations of glucose (A), insulin (C), total GIP (E), and TG (G) after the administration of glucose alone (○) or with TAG (●) or DAG (■) through gastric gavage in anesthetized male C57BL/6J mice. AUC are calculated from 0–120 min for glucose (B), from 0–30 min for insulin (D), and from 0–60 min for GIP (F). Data are expressed as means ± SEM; n = 7–9 in each group. Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were used when comparing TG values between TAG and DAG groups. *Asterisks* indicate the probability level of random differences between groups: **, $P < 0.01$. Statistical analysis was conducted using a one-way ANOVA and subsequently Fisher's PLSD multiple comparison. a–c, Means not sharing a given *letter* differ significantly ($P < 0.05$).

Blood glucose and insulin responses to DAG

The coadministration of DAG oil with glucose significantly increased the insulin level at 10 min, followed by a marked decline in the blood glucose at 30 min compared with glucose alone (Fig. 4, A and C). However, the peak insulin level after DAG was significantly lower than that after TAG administration (Fig. 4C). The insulin response for 30 min ($AUC_{Insulin}$30 min) was also significantly lower after DAG compared with TAG administration (Fig. 4D). The blood glucose response after DAG did not significantly differ from that after TAG administration (Fig. 4, A and B).

GIP and TG responses after DAG ingestion

The administration of DAG oil stimulated a postprandial GIP response either with or without glucose. However, the peak GIP level was significantly lower after DAG administration compared with that of TAG either with (Fig. 4E) or without glucose (Fig. 5A). The GIP response for 60 min (AUC_{GIP}60 min) was significantly lower after DAG administration (~20%, $P < 0.05$) compared with that of TAG either with or without glucose (Figs. 4F and 5B). The plasma TG response was also significantly lower after DAG administration compared with that of TAG either with or without glucose (Figs. 4G and 5C).

Effect of PL on fat-induced GIP secretion

Figure 6 shows the effect of PL on plasma GIP (Fig. 6, A and B) and the TG response (Fig. 6C) after TO administration. Either the peak GIP level (Fig. 6A) or GIP response for 60 min (~40% in AUC_{GIP}60 min, Fig. 6B) was significantly decreased by the addition of PL. The plasma TG response was completely inhibited by PL (Fig. 6C).

Discussion

The first finding in this study was that the co-ingestion of fat with glucose reduced the postprandial glycemic response. Even though the co-ingestion of fat did not affect peak blood glucose levels, it increased peak insulin levels and subsequently decreased blood glucose to lower levels. Blood glucose after 30 min was lower in the presence of fat and was negatively associated with the peak insulin level. There are several possible factors that

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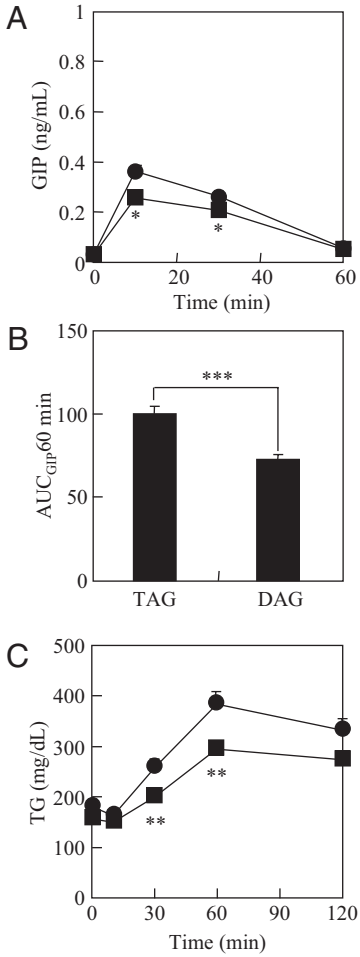

FIG. 5. Plasma concentrations of total GIP (A) and TG (C) after the administration of TAG (●) or DAG (■) (without glucose) through gastric gavage in anesthetized male C57BL/6J mice. AUC are calculated from 0–60 min for GIP (B). Data are expressed as means ± SEM; n = 10 in each group. Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were used when comparing values between the groups. *Asterisks* indicate the probability level of random differences between groups: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

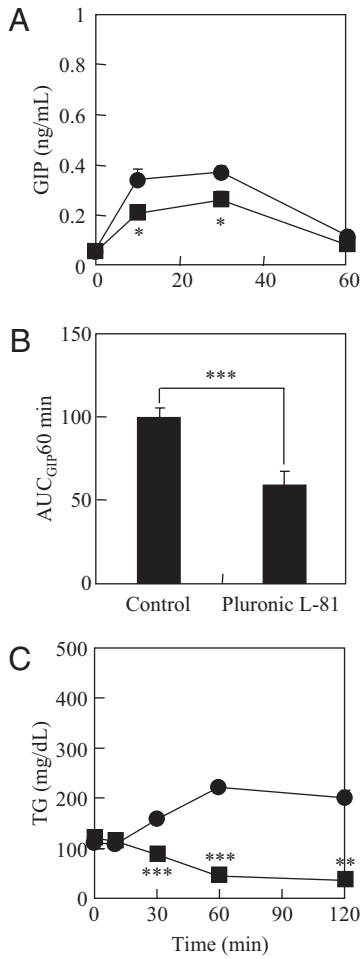

FIG. 6. Plasma concentrations of total GIP (A) and TG (C) after the administration of TO with (■) or without PL (●) through gastric gavage in anesthetized male C57BL/6J mice. AUC are calculated from 0–60 min for GIP (B). Data are expressed as means ± SEM; n = 10 in each group. Student's *t* tests after a preliminary F test of the homogeneity of within-group variance were used when comparing values between the groups. *Asterisks* indicate the probability level of random differences between groups: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

could have contributed to the lower glycemic response to glucose in the presence of fat. Although we cannot exclude the possibility that fat ingestion may alter the hepatic extraction of insulin, the most likely explanation is that dietary fat augments glucose-induced insulin secretion and thereby stimulates glucose uptake by insulin-targeted tissues. Consistent with this, the co-ingestion of fat increased glucose distribution in adipose tissue and skeletal muscle. An increased hepatic uptake of glucose was not observed after the co-ingestion of fat in this study.

We calculated total radioactivity in each tissue including expired gas at 120 min by taking account of the tissue weight. Total radioactivity was estimated to be approximately 7% less after glucose plus triolein than after glucose alone. More radioactivity was incorporated into the gastrocnemius muscle and epididymal fat tissues when glucose was administered with fat. Accordingly, the missing radioactivity after glucose plus triolein might be incorporated into other muscle and adipose tissues. However, we cannot exclude the possibility that more radioactivity remains in other tissues, *e.g.* the intestine.

The peak insulin levels after gastric glucose gavage varied markedly between experiments (ranging from 1.75–3.84 ng/ml), whereas the blood glucose response and the magnitude of stimulation of the insulin response by triolein appeared to be consistent. The mechanism responsible for generating the interexperimental variation in peak insulin levels remains unknown. Accordingly, fat-induced stimulation of the insulin response should be evaluated by comparison with a control (glucose alone).

It is known that gastric emptying contributes to postprandial glucose concentrations in healthy subjects and in patients with type 2 diabetes (32, 33). In this study, we did not study gastric emptying. However, it is unlikely that delayed gastric emptying is responsible for the acute changes in insulin secretion after the ingestion of fat observed in this study, because the peak blood glucose level did not differ between the experimental groups. The AUC for glucose and insulin may be affected by the reduced gastric emptying in the later phase of the study period.

One possible mechanism by which the potentiation of the insulin response to glucose could occur is via gut-derived incre-

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tins, GIP and GLP-1 (21). The addition of fat to glucose gave rise to a greatly increased GIP response at 10 min when fat-induced hyperinsulinemia was observed. In addition, the GIP-specific antagonist ANTGIP reduced fat-promoted insulin secretion but did not affect insulin secretion after the administration of glucose alone. Previous studies have shown that fat is a potent stimulus of GIP release, and GIP has been shown to potentiate glucose-induced insulin secretion (34–38). These results together indicate that fat-induced GIP secretion plays a significant role in the promotion of hyperinsulinemia after the co-ingestion of glucose and fat. However, the involvement of another mechanism cannot be excluded because GIP antagonism inhibited only half of the fat-promoted insulin secretion.

Tseng *et al.* (39) reported that ANTGIP treatment decreased insulin secretion after a glucose meal in rats. In this study, postprandial insulin secretion after glucose alone was not affected by ANTGIP treatment in mice. A few studies have reported the physiological concentration of GIP that stimulates glucose-induced insulin release. Siegel and Creutzfeldt (40) reported that GIP of 1–100 ng/ml (0.196–19.6 nM) enhanced glucose-induced insulin release by isolated rat pancreatic islets. Tseng *et al.* (28) showed that the glucose-induced insulin response was enhanced by iv GIP infusion (0.5–1.5 nmol/kg) in rats. In this study, the peak plasma GIP was approximately 0.270 ng/ml (0.054 nM) after glucose alone, whereas glucose plus triolein raised the peak GIP level up to 0.660 ng/ml (0.132 nM). Even though the physiological threshold of the GIP level still remains to be clarified, the lower GIP level after the administration of glucose alone did not appear to exhibit an insulinotropic effect in this study. The contribution of GIP to glucose-induced insulin secretion remains to be further studied.

GIP has been shown to stimulate glucose uptake via Akt activation (41) in adipocytes as well as insulin. Therefore, fat-induced GIP secretion may also contribute to rapid blood glucose elimination. The postprandial TG response was also significantly reduced in the presence of glucose compared with fat alone. A recent study also demonstrated that GIP, like insulin, activates lipoprotein lipase in adipocytes (42). One possibility to explain the reduced lipidemia after the co-ingestion of glucose and fat is increased TG uptake from the circulation by adipose tissues. However, the reduction of hepatic very-low-density lipoproteins output cannot be excluded.

In contrast to the immediate and marked increase in GIP after the ingestion of fat in combination with glucose, the administration of fat with glucose did not affect the active GLP-1 response at 10 min, and tended to increase the plasma GLP-1 level only at 30 min compared with that achieved by glucose alone. It is likely that GIP does but GLP-1 does not contribute to the fat-induced hyperinsulinemia observed at 10 min in this study. Ingested nutrients may have reached GIP-producing K cells in the upper small intestine within the 10-min study period, whereas a longer period of time is required to affect GLP-1-producing L cells in the distal intestine. The increased GLP-1 levels observed after 30 min in this study are consistent with previous studies demonstrating increased GLP-1 after fat-rich meal ingestion (43–45). Gunnarsson *et al.* (46) showed that active GLP-1 was increased by fatty acids at 15 min after gastric gavage. Similarly, previous *in vitro* studies have shown that fatty acids stimulate GLP-1 release in primary rat L cells and in a mouse L-cell line (47, 48), and the stimulation of GLP-1 secretion by fatty acids is mediated via the activation of G protein-coupled receptor 120 (49). Our inability to detect fat-mediated active GLP-1 release at 10 min *in vivo* may be because of a low concentration of fatty acids at the location of L cells after fat administration. It is also possible that fat-induced GLP-1 secretion is a slow phenomenon *in vivo*, possibly because the secretion of GLP-1 from ileal L cells is regulated by a complex proximal-distal loop that involves both GIP and neural factors (50). Another possible explanation for the lack of active GLP-1 stimulation in this study may be the low dose of glucose or triolein (2 mg/g BW each). Recently, Althage *et al.* (51) reported that active GLP-1 levels were very low after the oral administration of 3 mg glucose/g BW. Therefore, they administered a high dose of glucose (6 mg/g BW) or 3 mg/g glucose plus intralipid when active GLP-1 was to be measured. Finally, the rapid degradation of intact GLP-1 by the ubiquitous enzyme dipeptidyl peptidase IV cannot be excluded because we did not examine total GLP-1.

In this study, fat administration in combination with glucose augmented insulin levels and enhanced glucose elimination compared with glucose alone. This is consistent with a previous report showing that the fat content of the diet was negatively associated with the postprandial glycemic response in healthy volunteers (52). The lower glycemic response in the presence of fat may appear beneficial for the glycemic control of diabetics. However, the changes found after the co-ingestion of fat indicate at least a potentiation of insulin secretion that could form the basis of the insulin resistance associated with the chronic consumption of high-fat diets. The possibility that GIP plays a role in fat assimilation and the pathogenesis of obesity also cannot be excluded. Previous studies have shown that GIP increases lipoprotein lipase activity in preadipocytes (53), and the inhibition of GIP signaling prevents the onset of obesity and consequent insulin resistance induced by a high-fat diet (54). Thus, despite the apparent improvement in blood glucose levels that occurs when carbohydrate is ingested together with fat, the observation that the insulin levels were promoted suggests that increasing the fat content of meals would not be beneficial for obesity and diabetics.

Recent studies have shown that DAG-rich diets attenuate weight gain and improve glucose tolerance compared with a TAG-rich diet of a similar fatty acid composition in animals (55–58) and humans (59–61). In addition, DAG ingestion reduced postprandial lipidemia in humans (62, 63). These effects of dietary DAG are probably caused by the different metabolic fates due to the variation in the structures of the lipids. However, the effect of DAG on postprandial GIP secretion has not been elucidated.

Of greater importance is the novel finding that DAG stimulated less GIP secretion either with or without glucose compared with TAG of a similar fatty acid composition. DAG also stimulated a lower insulin response compared with TAG in combination with glucose. These results support the previous studies indicating that the postprandial insulin response was lower in DAG than TAG when dietary oil was ingested in combination with carbohydrate (26, 27). Despite the lack of a change in the activity of GIP or active GIP secretion, this study suggests the

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intriguing possibility that dietary DAG stimulates a lower GIP release in the postprandial state and thereby a reduced insulin response compared with TAG of a similar fatty acid composition.

Because very little is known about the apparent mechanism of fat-induced GIP secretion, it is still unclear how DAG stimulates reduced GIP secretion compared with TAG. A previous study showed that the intragastric infusion of an emulsion containing DAG significantly inhibited the lymphatic transport of TG as chylomicrons in rats (64). To investigate whether fat-induced GIP secretion is associated with chylomicron formation, we studied the effect of PL, an inhibitor of chylomicron formation, on GIP secretion after TO administration. Previous reports indicated that PL inhibits chylomicron secretion into the lymph (65, 66) without affecting the intestinal digestion, absorption, and re-esterification of TG (67, 68). Tso *et al.* (66) suggested that PL blocked either the formation of chylomicrons or the movement of these particles out of the endoplasmic reticulum. In this study, PL significantly attenuated the fat-induced plasma TG and GIP response throughout the study period. This suggests that fat-induced GIP secretion is associated with chylomicron formation in the intestinal mucosa. Decreased GIP secretion after DAG ingestion may be attributed to retarded chylomicron formation. Morita *et al.* (69) proposed that PL inhibited the secretion of chylomicrons by leading the excess core expansion of the primordial lipoproteins and the conformational modification of apolipoprotein B. However, the molecular mechanism behind the relationship between GIP secretion and the intracellular metabolism of fat in K cells still remains to be elucidated.

In conclusion, we present evidence that fat ingestion in combination with glucose increases GIP secretion compared with glucose alone. This conclusion is limited by the fact that GIP release has been shown to be directly proportional to calorie intake. Thus, the higher calorie content of test meals containing both carbohydrate and fat compared with test meals containing carbohydrate alone may contribute to superior GIP release. This results in augmented insulinemia, stimulated glucose, and lipid storage in adipose tissues and possibly inhibits lipolysis. The results of the present study suggest that nutrients may serve as exogenous physiological regulators of hormone secretion with influences on glucose and lipid homeostasis. The results also suggest that oral fat ingestion differentially affects postprandial GIP secretion while still leading to a different insulin level, possibly indicating that dietary fat may differentially regulate postprandial energy metabolism via hormones. This would be of importance for the further development of incretin-based therapy for the treatment of obesity and related diseases. Therefore, more detailed studies of incretin hormone kinetics should be undertaken in relation to energy homeostasis in larger animals and humans.

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Address all correspondence and requests for reprints to: Akira Shimotoyodome, Ph.D., 2606 Akabane, Ichikai-machi, Haga-gun, Tochigi 321-3497, Japan. E-mail: shimotoyodome.akira@kao.co.jp.

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