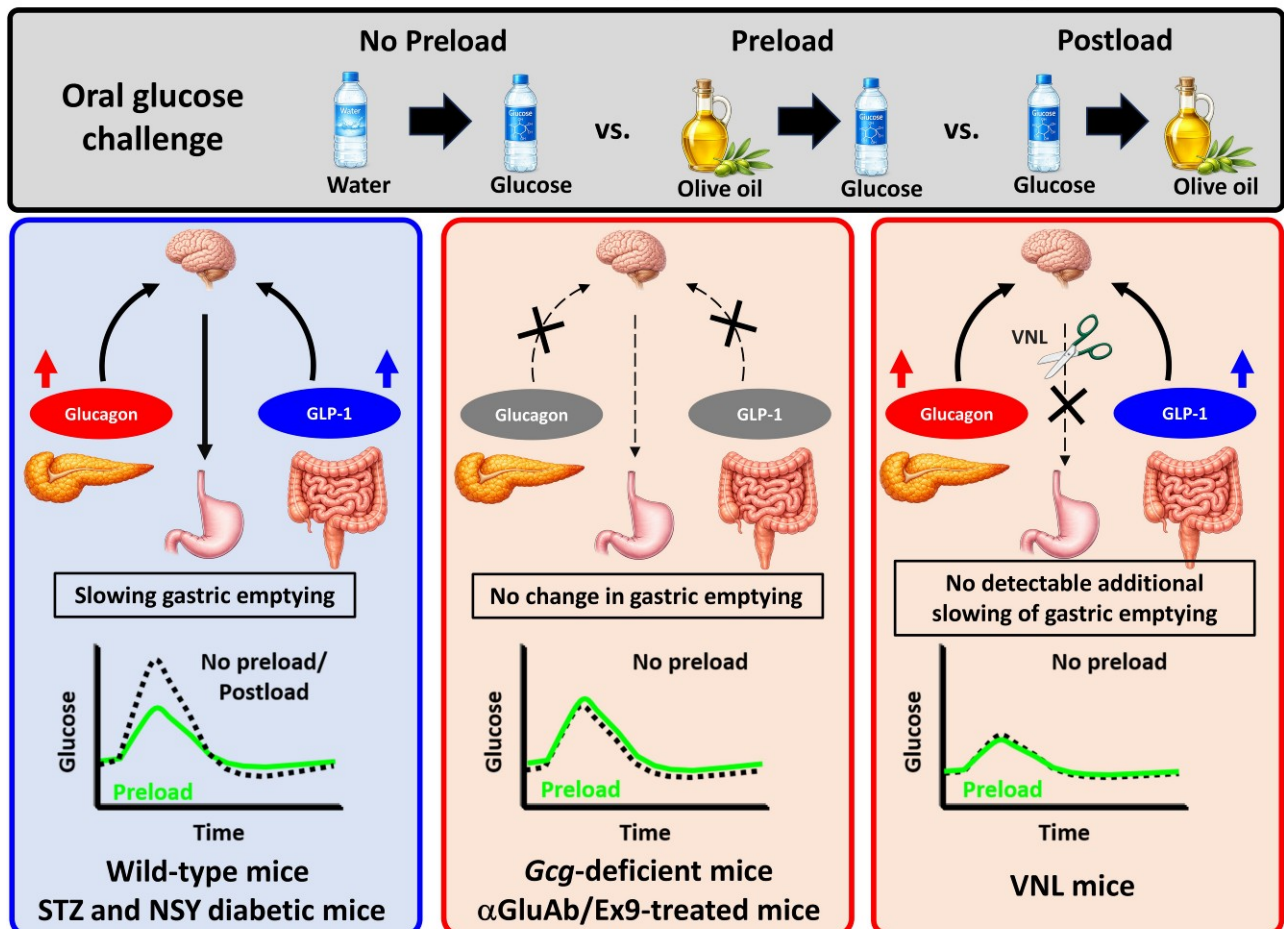


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Diabetes 2026;75(12):1–12 | <https://doi.org/10.2337/db26-0531>

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αGluAb, monoclonal anti-glucagon receptor antibody; EX9, exendin(9-39); GLP-1, glucagon-like peptide-1; STZ, streptozotocin; VNL, vagus nerve ligation.



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Consumption of fat before carbohydrate (preload) attenuates postprandial glucose excursions, although the underlying mechanisms remain unclear. We investigated whether an olive oil preload (OG) reduces glycemia by engaging endocrine and neural regulation of gastric emptying. Wild-type C57BL/6J mice received OG or olive oil after (GO) an oral glucose load, with a water preload (WG) as control. Glucose, insulin, glucagon, glucose-dependent insulintropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and gastric emptying were assessed. Studies were also performed in streptozotocin-induced diabetic mice, NSY diabetic mice, *Gcg*-deficient and *Glp1r*-deficient mice, mice receiving glucagon and/or GLP-1 receptor blockade, and mice subjected to bilateral subdiaphragmatic vagus nerve ligation (VNL). Compared with WG and GO, OG reduced the early glucose excursion and slowed gastric emptying, accompanied by increased glucagon, GIP, and GLP-1 secretion. Similar effects were evident in diabetic mice. The response was absent in *Gcg*-deficient mice but largely preserved in *Glp1r*-deficient mice and following blockade of either receptor alone. Combined receptor blockade attenuated glucose lowering and eliminated detectable gastric slowing, supporting partially redundant signaling. VNL attenuated these effects, despite preserved or enhanced hormone

ARTICLE HIGHLIGHTS

- We undertook this study to define how fat consumed before oral glucose attenuates postchallenge glycemia.
- We asked whether olive oil reduces glycemia by slowing gastric emptying and investigated contributing endocrine and neural pathways.
- Olive oil increased glucagon, glucose-dependent insulintropic polypeptide, and glucagon-like peptide-1; slowed gastric emptying; and reduced early glycemia in mice. Glucagon and glucagon-like peptide-1 signaling acted redundantly, with vagal involvement.
- Nutrient timing integrates endocrine-neural regulation of gastric emptying and glycemia, supporting evaluation of meal sequencing in humans.

secretion, supporting a contribution of vagal signaling. These findings identify nutrient timing as an integrated endocrine-neural regulator of postchallenge blood glucose homeostasis.

More than 580 million people worldwide were living with diabetes, predominantly type 2 diabetes (T2D), in 2025,

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and this number is projected to increase to 853 million by 2050 (1). Lifestyle interventions, including dietary modification and increased physical activity, are effective in preventing the development and progression of T2D (2,3). However, long-term adherence to such interventions is poor in the majority of individuals (4,5), underscoring the need for simple and sustainable dietary strategies. When glycemic control is reasonable (i.e., glycosylated hemoglobin [HbA_{1c}] is <8.0% [64 mmol/mol]), the postprandial, rather than fasting, glucose is the major determinant of average glycemic control as assessed by HbA_{1c} (6). Accordingly, postprandial glucose is an important therapeutic target.

Meal sequence has emerged as a practical approach for improving postprandial glucose control in people with prediabetes or T2D (7,8). Consumption of vegetables before carbohydrate-rich foods attenuates glucose excursions (9), likely through delayed intestinal carbohydrate absorption mediated by dietary fiber. Similarly, consumption of protein- and fat-rich foods before carbohydrate intake reduces postprandial glycemic excursions (10–12). These effects are thought to be mediated, at least in part, by the interaction of nutrients with the small intestine to induce the secretion of glucagon-like peptide-1 (GLP-1) and slowing of gastric emptying (10–12). The slowing of gastric emptying by fat is known to depend on its digestion to fatty acids and their chain length (13).

Gastric emptying is a major determinant of postprandial glycemia in both health and T2D, exhibits substantial intra- and interindividual variation, and is regulated by integrated neural and hormonal pathways, including GLP-1, cholecystokinin (CCK), and glucagon (14–17). There is also evidence that vagovagal neural circuits play a central role in coordinating gastric motility and that metabolic

disturbances, including acute hyperglycemia, can differentially modulate gastric emptying (18). Despite these observations, the relative contributions of these peptides and neural pathways to the delay in gastric emptying induced by protein or fat consumed before carbohydrate remain poorly defined. By inference, this information is of major relevance to dietary strategies to diminish postprandial glycemic excursions.

The metabolic and gastrointestinal effects of dietary fat depend not only on the amount consumed but also on fatty acid composition (19). Different dietary oils variably stimulate gut hormone secretion and slow gastric emptying, with long-chain unsaturated fatty acids generally exerting greater effects than saturated or medium-chain fatty acids (13). Accordingly, the glucose-lowering efficacy of a fat preload may vary according to the type of dietary fat. In this study, we evaluated the effects of olive oil administration before, versus after, an oral glucose load in several mouse models and investigated the endocrine and neural mechanisms linking nutrient sequence, gastric emptying, and glycemia.

RESEARCH DESIGN AND METHODS

Animal Experiments

All animal experiments were approved by the Animal Care Committee of Gifu University Graduate School of Medicine (2021-93) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Five-week-old male C57BL/6J mice and 8-week-old male NSY mice were obtained from Japan SLC (Shizuoka, Japan). Streptozotocin (STZ)-induced diabetic mice were generated by a single intraperitoneal injection of STZ (150 mg/kg) in 7-week-old C57BL/6J mice after a 16-h fast. Mice with blood glucose >300 mg/dL 1 week later were used. CCK-A receptor (*Cckar*)-deficient

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Received 18 June 2026 and accepted 27 August 2026

This article contains supplementary material online at <https://doi.org/10.2337/figshare.33357363>.

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mice were provided by RIKEN Bioresource Center (20). *Gcg*-deficient mice and *Glp1r*-deficient mice were generated by Y.Ha. and D.J.D., respectively, as previously described (21,22). Mice were housed under a 12-h light/dark cycle at 23°C with free access to water and an autoclaved CE-2 diet (CLEA Japan, Tokyo, Japan).

Oral Glucose Tolerance Test With Preloading or Postloading of Various Oils

Eight-week-old mice were used in these experiments. Following an overnight fast, mice were randomly assigned to experimental groups. In the olive oil preload (OG) group, olive oil (5 mL/kg body weight; Ajinomoto Co., Inc., Tokyo, Japan) was orally administered 15 min before an oral glucose tolerance test (OGTT) (2 g/kg [4 mL/kg] body weight). In the olive oil postload (GO) group, olive oil was administered 15 min after glucose loading. In the control group, water was administered before glucose loading (WG). The time of oral glucose administration during the OGTT was defined as 0 min. Blood samples were collected from the tail vein at −15, 0, 15, 30, 60, and 120 min relative to glucose administration. Blood glucose levels were measured using a FreeStyle Freedom Light monitoring system (cat. no. 121-30854000; Nipro, Osaka, Japan). Blood samples were placed on ice and centrifuged at 800g for 10 min at 4°C to separate plasma. Plasma samples were either analyzed immediately or stored at −80°C until further processing.

In addition to olive oil, preload experiments were performed using coconut oil (Brown Sugar 1st Co., Ltd., Tokyo, Japan), lard (Megmilk Snow Brand Co., Ltd., Tokyo, Japan), fish oil (Carlson Laboratories, Inc., Lincolnshire, IL), and flaxseed oil (The Nisshin OilliO Group, Ltd., Tokyo, Japan). A monoclonal anti-glucagon receptor antibody (α GluAb) (4 mg/kg) and a control antibody (obtained from Novo Nordisk) were administered 2 h before glucose loading. Exendin(9-39) (Ex9), a GLP-1 receptor antagonist (25 nmol/kg; cat. no. ab141101; Abcam, Cambridge, U.K.), was administered 1 h before glucose loading (8). Control animals received vehicle (0.9% NaCl [10 mL/kg]).

Measurement of Hormones and Gastric Emptying

Insulin, total glucose-dependent insulinotropic polypeptide (GIP), total GLP-1, and glucagon levels were measured by ELISA using the following kits: Ultra PLUS Sensitive Mouse Insulin ELISA Kit (cat. no. M1105; Morinaga, Tokyo, Japan), Rat/Mouse GIP (Total) ELISA (cat. no. EZRMGIP-55 K; Merck Millipore, Darmstadt, Germany), V-PLEX GLP-1 (Total) Kit (cat. no. K1503PD; Meso Scale Discovery, Rockville, MD), and Glucagon ELISA Kit (cat. no. 10-1281-01; Mercodia AB, Uppsala, Sweden).

Acute gastric emptying experiments were conducted in the morning after a 16-h fast. Acetaminophen (100 mg/kg body weight, cat. no. A161220; Toronto Research Chemicals Inc., Toronto, Canada) was administered together with glucose at time 0 min. Blood samples were collected from

the tail vein at 0, 15, 30, 60, and 120 min. Acetaminophen concentrations were measured in duplicate using a commercially available assay kit (cat. no. 132419; Neogen Corporation, Lansing, MI).

Vagus Nerve Ligation

Bilateral subdiaphragmatic vagus nerve ligation (VNL) was performed in male C57BL/6J mice as previously described (23). Under isoflurane anesthesia, a midline abdominal incision was made to expose the upper abdominal organs. The bilateral subdiaphragmatic vagal trunks running along the esophagus were carefully exposed and individually ligated using 5-0 sutures. In sham-operated mice, the vagal trunks were similarly exposed, but not ligated. VNL and sham-operated mice were housed in separate cages. Two weeks after surgery, OGTT was performed. At the end of the experiments, successful bilateral subdiaphragmatic VNL was confirmed by increased stomach weight, a well-established phenotype of bilateral subdiaphragmatic VNL (23).

Statistical Analysis

Data are presented as mean $\pm$ SEM. Statistical analyses and graph generation were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA). Comparisons used one- or two-way ANOVA followed, as appropriate, by Tukey or Dunnett post hoc test. $P < 0.05$ was considered statistically significant.

Data and Resource Availability

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. All unique reagents and animal models generated in this study are available from the corresponding author upon reasonable request, subject to applicable institutional and material transfer agreements.

RESULTS

OG Suppresses Postchallenge Glucose Excursions and Slows Gastric Emptying

To investigate the mechanistic basis of meal sequence-induced improvement in postprandial glycemia, we established a mouse model in which olive oil was administered either before (OG) or after (GO) oral glucose loading, with a water preload as the control (WG). OG reduced glucose levels at 15 and 30 min and the glucose area under the curve (AUC) from 0 to 60 min compared with WG and GO (Fig. 1A). The glucose AUC from 0 to 120 min did not differ significantly among the groups, indicating that the effect was predominantly early and not associated with late rebound hyperglycemia. Plasma insulin and AUC of insulin (AUC-insulin) did not differ among groups (Fig. 1B), whereas OG enhanced plasma glucagon, GIP, and GLP-1 responses, particularly early after glucose loading (Fig. 1C–E). Although glucagon declined between −15 and 0 min within OG, it remained

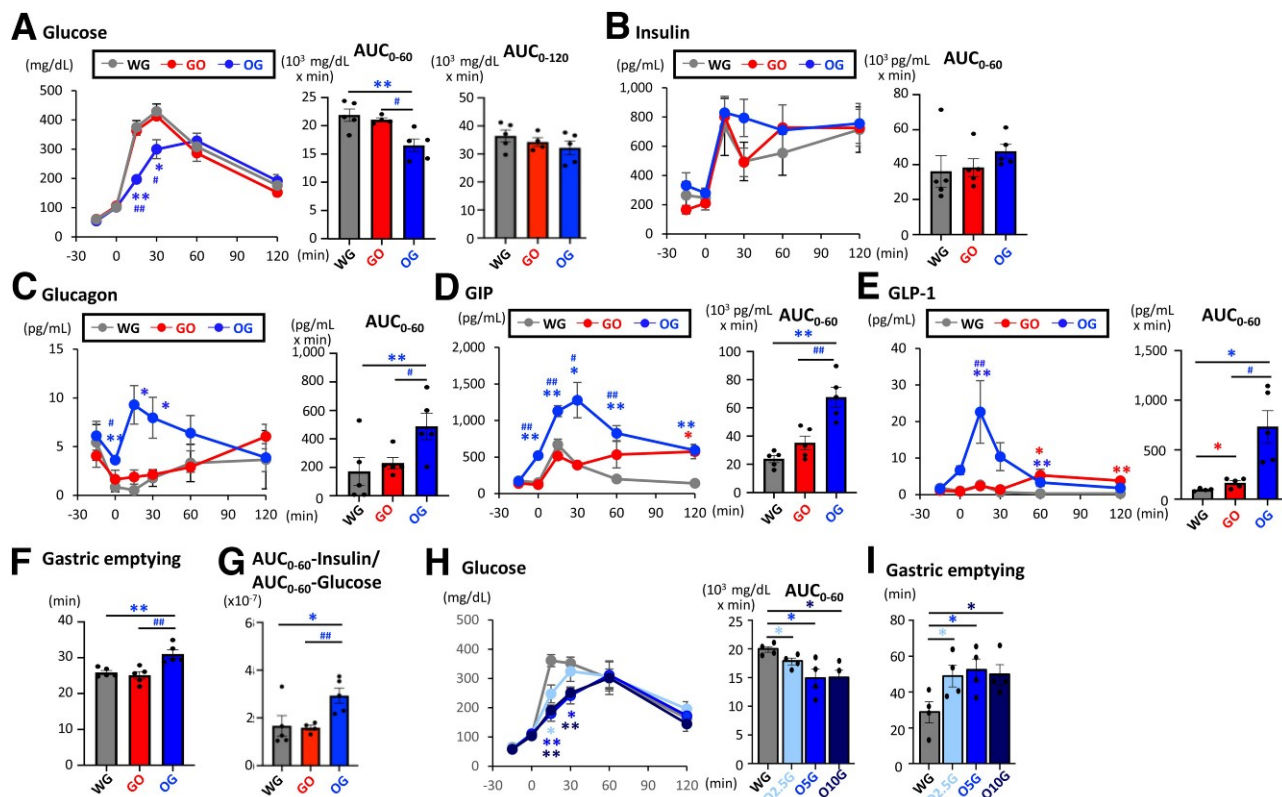


Figure 1—Effects of OG and GO on postchallenge glucose excursion, hormone secretion, and gastric emptying in wild-type C57BL/6J mice. *A–E*: Time course changes and AUCs for blood glucose, insulin, glucagon, GIP, and GLP-1 in mice receiving WG, GO, or OG. *F*: Gastric emptying assessed as T50%, defined as the time at which 50% of the cumulative plasma appearance of acetaminophen is observed. *G*: AUC-insulin/AUC-glucose ratio. *H* and *I*: Effects of increasing olive oil volume on blood glucose excursions and gastric emptying: WG (water 10 mL/kg), O2.5G (olive oil 2.5 mL/kg + water 7.5 mL/kg), O5G (olive oil 5 mL/kg + water 5 mL/kg), and O10G (olive oil 10 mL/kg). Data are mean $\pm$ SEM ($n = 5$ –6 per group). * $P < 0.05$, ** $P < 0.01$ vs. WG; # $P < 0.05$, ## $P < 0.01$ vs. GO. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs. AUC₀₋₆₀, area under the curve from 0 to 60 min; AUC₀₋₁₂₀, area under the curve from 0 to 120 min.

higher than in WG immediately before glucose. OG prolonged the calculated gastric emptying half-time (Fig. 1*F*). The AUC-insulin/AUC-glucose ratio was significantly increased in OG mice (Fig. 1*G*), indicating maintained insulin secretion relative to glycemic exposure. OG at 2.5, 5, and 10 mL/kg dose dependently reduced early glucose excursions and slowed gastric emptying, with effects plateauing at 5 mL/kg (Fig. 1*H* and *I*); 5 mL/kg was therefore used subsequently.

Different Dietary Oils Exert Distinct Effects on Postchallenge Responses

We next compared the effects of several dietary oils (Fig. 2). A flaxseed oil preload (FLG), like OG, reduced both postchallenge glucose excursions and slowed gastric emptying, whereas a fish oil preload (FIG) had minimal effect (Fig. 2*A* and *F*). Glucagon responses to FLG and FIG were modest and remained lower than those in OG (Fig. 2*C*). GIP levels were comparable among FLG, FIG, and WG, whereas GLP-1 responses were increased in FLG and FIG relative to WG, although less prominently than in OG (Fig. 2*D* and *E*). Gastric emptying was significantly delayed in FLG and OG but not in FIG (Fig. 2*F*). The AUC-

insulin/AUC-glucose ratio was significantly increased by OG and to a lesser degree by FLG, whereas FIG had minimal effects (Fig. 2*G*). Similarly, among saturated fat-rich oils, a lard preload (LaG) significantly suppressed postchallenge glucose excursions, whereas a coconut oil preload (CG) had weaker effects (Fig. 2*H*). Glucagon response was modestly increased by both CG and LaG compared with WG, but the response remained less than that observed with OG (Fig. 2*J*). The GIP response was enhanced by LaG and, to a lesser extent, by CG, whereas OG elicited the greatest increase (Fig. 2*K*). Likewise, GLP-1 responses were augmented by both OG and LaG, with LaG having a greater effect than CG, although both were less potent than OG (Fig. 2*L*). Gastric emptying was delayed by LaG, CG, and OG (Fig. 2*M*). The AUC-insulin/AUC-glucose ratio was significantly increased by OG and, to a lesser degree, by LaG and CG (Fig. 2*N*).

OG Lowers Postchallenge Glucose in Diabetic Mouse Models

To determine whether the effects of an oil preload are preserved under diabetic conditions, experiments were

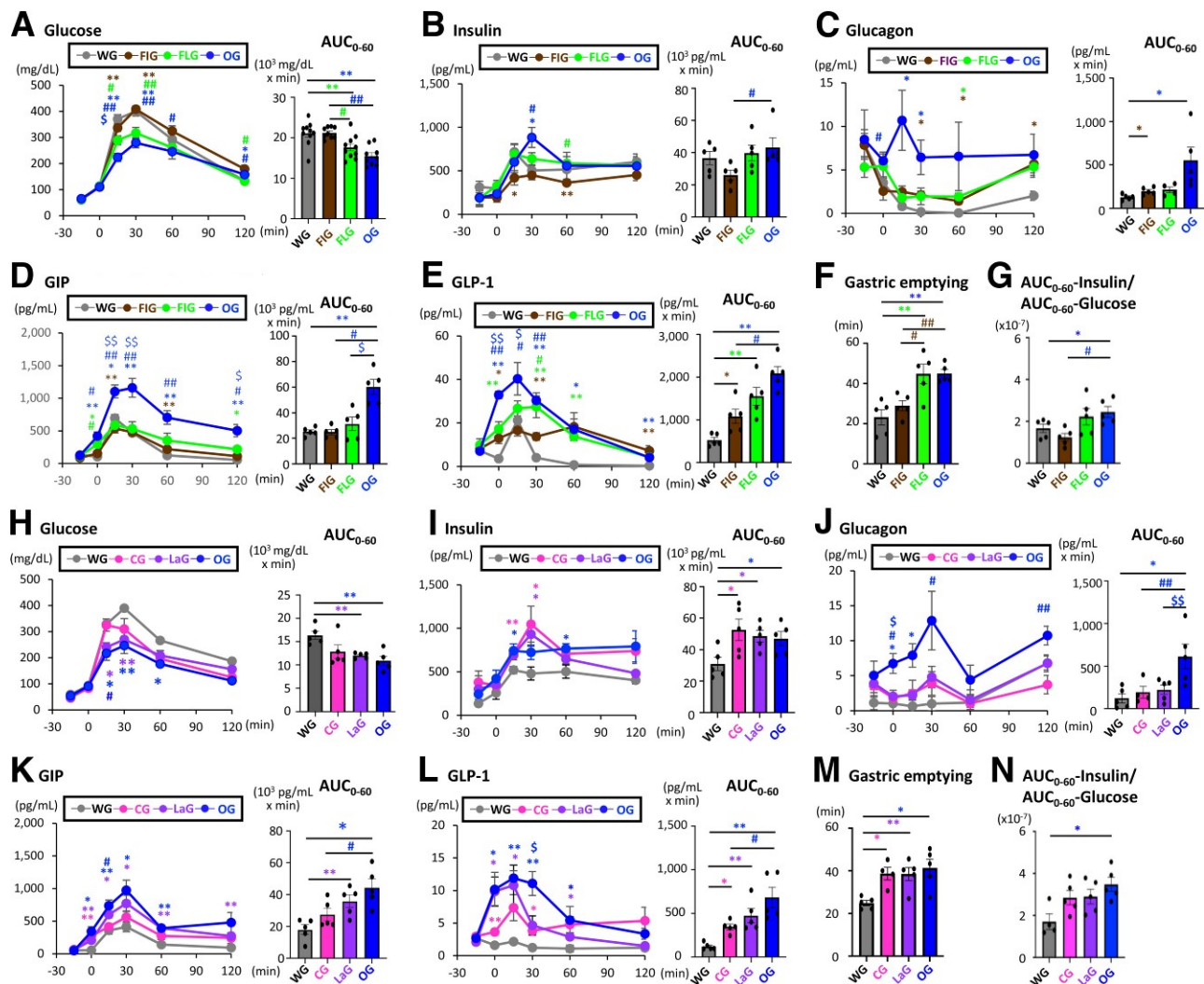


Figure 2—Effects of various oil preloads on postprandial glucose excursion, hormone secretion, and gastric emptying in wild-type C57BL/6J mice. **A–E**: Time course changes and AUCs for blood glucose, insulin, glucagon, GIP, and GLP-1 in mice receiving WG, FIG, FLG, or OG. **F**: Gastric emptying assessed as T50%, defined as the time at which 50% of the cumulative plasma appearance of acetaminophen is observed. **G**: AUC-insulin/AUC-glucose ratio. **H–L**: Time course changes and AUCs for blood glucose, plasma insulin, glucagon, GIP, and GLP-1 in mice receiving WG, CG, LaG, or OG. **M**: Gastric emptying assessed as T50%. **N**: AUC-insulin/AUC-glucose ratio. Data are mean $\pm$ SEM ($n = 5$ –6 per group). * $P < 0.05$, ** $P < 0.01$ vs. WG; # $P < 0.05$, ## $P < 0.01$ vs. FIG or CG; \$ $P < 0.05$, \$\$ $P < 0.01$ vs. FLG or LaG. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs. AUC₀₋₆₀, area under the curve from 0 to 60 min.

conducted in both STZ-induced diabetic mice (Fig. 3A–G) and diabetic NSY mice (Fig. 3H–N). In both models, OG reduced postchallenge glucose excursions and slowed gastric emptying compared with WG and GO (Fig. 3A, F, H, and M). In STZ-induced diabetic mice, circulating insulin levels were markedly reduced (Fig. 3B). Nevertheless, OG stimulated glucagon, GIP, and GLP-1 responses compared with GO, particularly during the early postprandial phase. In contrast, GO delayed hormonal responses in STZ-induced diabetic mice, a pattern distinct from that observed in wild-type mice (Fig. 1C–E). In NSY mice, insulin and AUC-insulin did not differ significantly among groups (Fig. 3I). However, OG enhanced glucagon, GIP, and GLP-1 responses compared with GO (Fig. 3J–L), whereas

GO failed to augment these hormonal responses, consistent with the findings in wild-type mice (Fig. 1C–E). Similarly, the AUC-insulin/AUC-glucose ratio was significantly increased by OG (Fig. 3N).

Partially Redundant Contributions of Glucagon and GLP-1 Receptor Signaling to the Effects of OG

Because both glucagon and GLP-1 may slow gastric emptying and because they are stimulated by preload administration of several oils, we hypothesized that glucagon and/or GLP-1 play a critical role in mediating the effects of an oil preload on postchallenge glycemia. To clarify the contribution of glucagon and GLP-1 receptor signaling to the metabolic effects of OG, we examined postchallenge responses

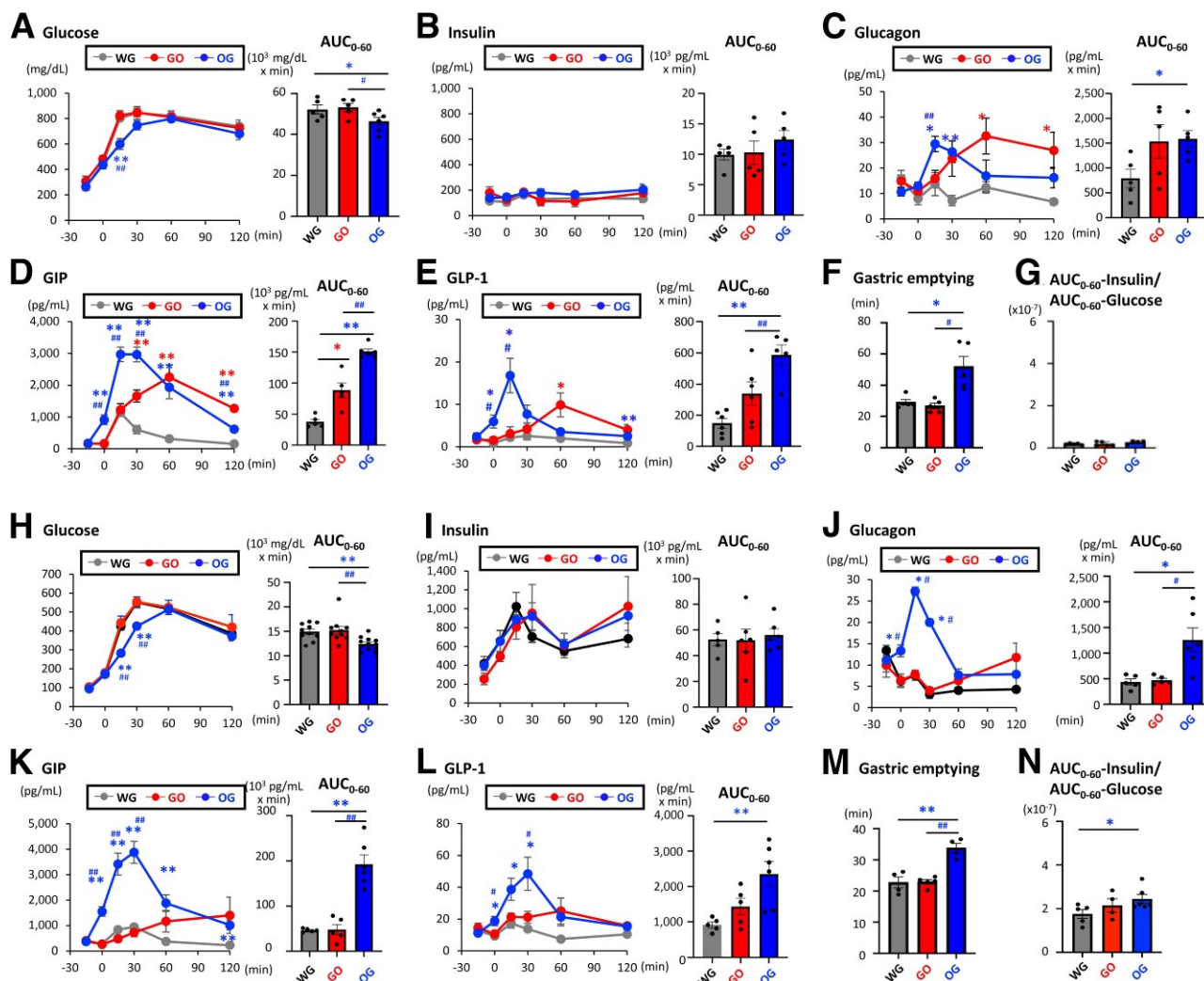


Figure 3—Effects of OG and GO on postchallenge glucose excursion, hormone secretion, and gastric emptying in diabetic mouse models. A–E: Time course changes and AUCs for blood glucose, insulin, glucagon, GIP, and GLP-1 in high-dose STZ-induced diabetic mice receiving WG, GO, or OG. **F:** Gastric emptying assessed as T50%, defined as the time at which 50% of the cumulative plasma appearance of acetaminophen is observed in STZ-induced diabetic mice. **G:** AUC-insulin/AUC-glucose ratio. **H–L:** Time course changes in blood glucose, plasma insulin, glucagon, GIP, and GLP-1 in diabetic NSY mice receiving WG, GO, or OG. **M:** Gastric emptying assessed as T50% in diabetic NSY mice. **N:** AUC-insulin/AUC-glucose ratio. Data are mean ± SEM ($n = 5-6$ per group). * $P < 0.05$, ** $P < 0.01$ vs. WG; # $P < 0.05$, ### $P < 0.01$ vs. GO. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs. AUC₀₋₆₀, area under the curve from 0 to 60 min.

in *Gcg*-deficient and *Glp1r*-deficient mice (Fig. 4). In *Gcg*-deficient mice, which lack multiple proglucagon-derived peptides, OG did not reduce the glucose excursion or slow gastric emptying, whereas both effects occurred in control mice (Fig. 4A and B). Both insulin and GIP levels following WG were higher in *Gcg*-deficient than in control mice (Supplementary Fig. 1A and B). OG enhanced GIP secretion significantly in control mice, and a similar trend was observed in *Gcg*-deficient mice, which did not achieve statistical significance. In contrast, in *Glp1r*-deficient mice, the effects of OG on glucose excursion and gastric emptying were essentially preserved, although the reduction in AUC-glucose did not achieve statistical significance (Fig. 4C and D). OG markedly enhanced glucagon, GIP, and GLP-1 responses, whereas WG had no effect (Supplementary Fig. 1E–G).

We next evaluated the contributions of glucagon and GLP-1 receptor signaling to the effects of OG using α GluAb and/or Ex9. The effects of OG on glucose excursions and gastric emptying were largely preserved in mice pretreated with either α GluAb or Ex9 alone (Fig. 4E–H and Supplementary Figs. 2 and 3). Simultaneous treatment with α GluAb and Ex9 attenuated the glucose-lowering effect of OG and eliminated its effect on gastric emptying (Fig. 4I and J and Supplementary Fig. 4). These findings support the concept of partially redundant contributions of glucagon and GLP-1 receptor signaling. Blockade of either pathway alone was insufficient to eliminate the response, whereas simultaneous blockade attenuated the glucose-lowering effect and eliminated the detectable delay in gastric emptying. Because CCK regulates lipid-induced gastric motility, we also examined the effects of OG in

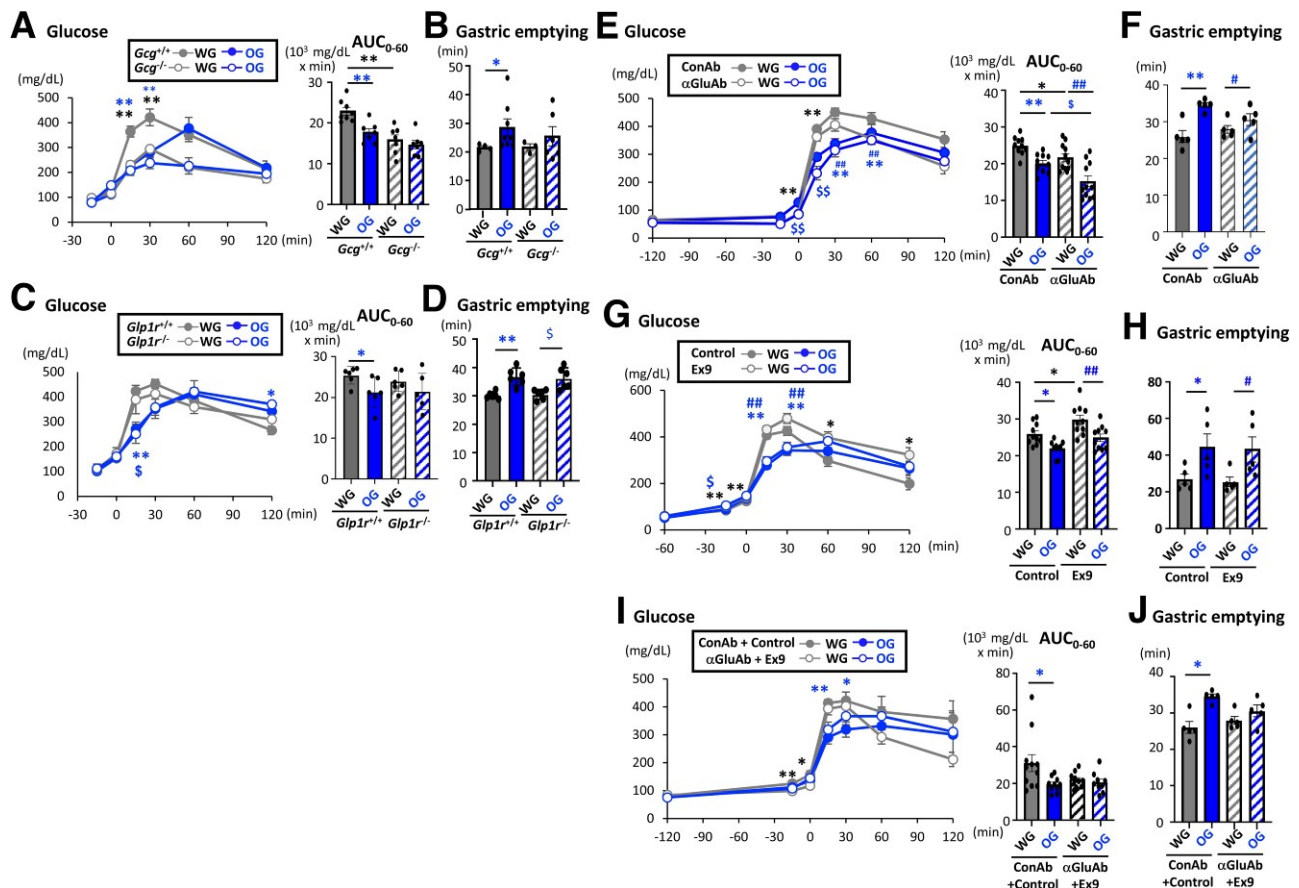


Figure 4—Genetic and pharmacological evidence for partially redundant contributions of glucagon and GLP-1 receptor signaling to the metabolic effects of OG. **A–D:** Time course changes and AUCs for blood glucose (**A** and **C**) and gastric emptying assessed as T50%, defined as the time at which 50% of the cumulative plasma appearance of acetaminophen is observed (**B** and **D**) in mice lacking glucagon-derived peptides (*Gcg*^{-/-}) or the GLP-1 receptor (*Glp1r*^{-/-}) and their littermate controls following WG or OG. Data are mean ± SEM (*n* = 5–6 per group). **P* < 0.05, ***P* < 0.01 vs. littermate control WG; \$*P* < 0.05 vs. *Gcg*^{-/-} mice or *Glp1r*^{-/-} mice WG. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs. **E–J:** Time course changes and AUCs for blood glucose (**E**, **G**, and **I**) and gastric emptying assessed as T50% (**F**, **H**, and **J**) in mice receiving WG or OG following treatment with Ex9 or saline (control) administered 1 h before glucose loading, αGluAb or control antibody (ConAb) administered 2 h before glucose loading, or combined αGluAb + Ex9 or ConAb + control. Data are mean ± SEM (*n* = 5–6 per group). **P* < 0.05, ***P* < 0.01 vs. ConAb WG, control WG, or ConAb + control WG; #*P* < 0.05, ##*P* < 0.01 vs. αGluAb WG, Ex9 WG, or αGluAb + Ex9 WG; \$*P* < 0.05, \$\$\$*P* < 0.01 vs. ConAb OG, control OG, or ConAb + control OG. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs. AUC₀₋₆₀, area under the curve from 0 to 60 min.

Cckar-deficient mice. OG reduced early glucose excursions and delayed gastric emptying similarly in *Cckar*-deficient and control mice (Supplementary Fig. 5), indicating that CCK-A receptor signaling was not indispensable in this setting.

Vagal Signaling Contributes to the Effects of OG

Because glucagon and GLP-1 are known to exert part of their biological effects through activation of vagal afferent pathways and because vagal signaling plays an important role in the regulation of gastric emptying, we hypothesized that vagal pathways contribute to the effects of an oil preload on glucose excursions and gastric emptying. To evaluate this, we assessed the effects of bilateral subdiaphragmatic VNL (Fig. 5). In sham-operated mice, OG reduced post-challenge glucose excursion and slowed gastric emptying,

whereas in VNL mice, these effects were attenuated or abolished (Fig. 5A and F). In contrast, OG-induced increases in glucagon, GIP, and GLP-1 responses were preserved or enhanced after VNL (Fig. 5C–E). These findings support a contribution of vagal signaling to the regulation of glucose excursions and gastric emptying by OG.

DISCUSSION

This study demonstrates that OG reduces early glucose excursion and slows gastric emptying in mice, accompanied by increases in glucagon, GIP, and GLP-1. These responses were preserved in STZ-induced insulin-deficient diabetic mice and NSY diabetic mice, indicating that the effects of an oil preload are preserved even when β-cell function is impaired. Genetic and pharmacological findings support partially redundant contributions of glucagon and GLP-1

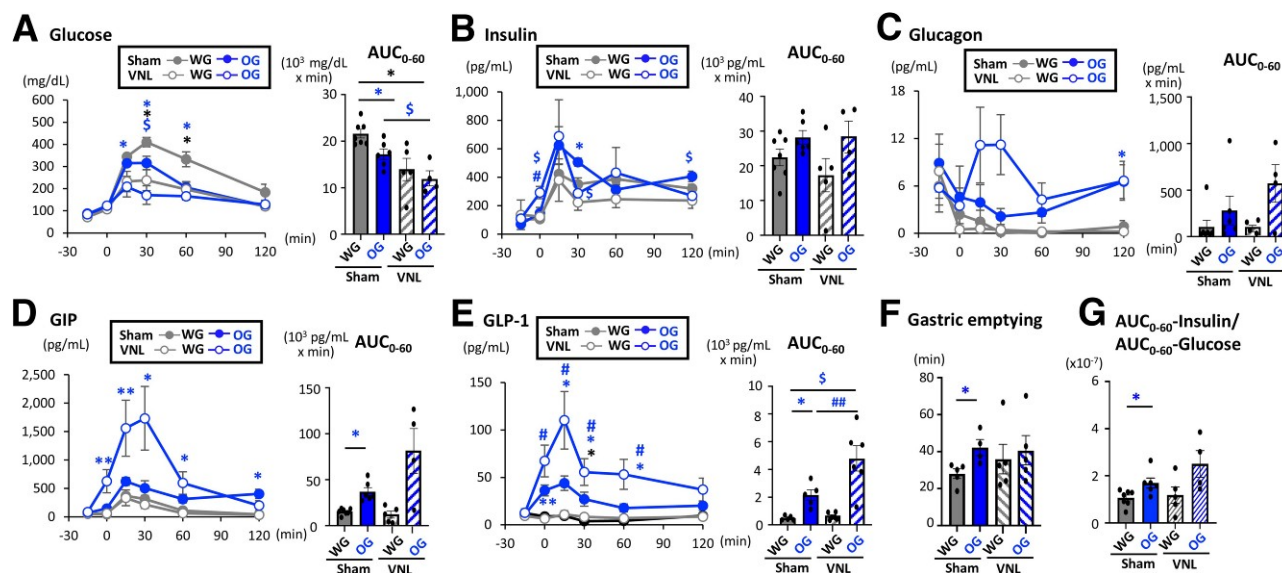


Figure 5—Effects of OG in mice with VNL. A–E: Time course changes and AUCs for blood glucose, insulin, glucagon, GIP, and GLP-1 in sham-operated or VNL mice receiving WG or OG. F: Gastric emptying assessed as T50%, defined as the time at which 50% of the cumulative plasma appearance of acetaminophen is observed. G: AUC-insulin/AUC-glucose ratio. Data are mean $\pm$ SEM ($n = 5$ –6 per group). * $P < 0.05$, ** $P < 0.01$ vs. sham WG; # $P < 0.05$, ## $P < 0.01$ vs. VNL WG; \$ $P < 0.05$ vs. sham-OG. Symbols indicating statistical significance are color-coded to match the corresponding groups shown in the graphs.

receptor signaling, while the outcomes of VNL experiments support a contribution of vagal pathways. Thus, the effect appears to involve an integrated endocrine-neural response rather than a single hormone.

Meal sequence is a practical strategy for reducing postprandial glycemic excursions, with consumption of vegetables, protein, or fat before carbohydrates shown to attenuate postprandial glucose responses (7,24). These effects have been attributed to delayed glucose absorption and enhanced incretin secretion, particularly GLP-1 (10,25). Our findings extend this concept by demonstrating that the timing of fat exposure is a critical determinant of enteroendocrine activation. OG, but not GO elicited early glucagon, GIP, and GLP-1 responses and reduced early glycemic excursions. These observations suggest that early nutrient sensing activates coordinated endocrine responses that contribute to slowing gastric emptying and limiting glucose excursions.

The metabolic effects of dietary fats vary according to their fatty acid composition, chain length, and degree of saturation, which may influence enteroendocrine hormone secretion and gastric emptying (13,19). Flaxseed oil resembled olive oil, whereas fish oil had little effect; lard elicited stronger responses than coconut oil. The difference between flaxseed and fish oils cannot be explained by chain length alone and may reflect their distinct fatty acid profiles. Flaxseed oil is rich in α -linolenic acid, whereas marine fish oils characteristically contain eicosapentaenoic acid and docosahexaenoic acid (Supplementary Table 1). Differences in digestion, micellar incorporation, intestinal availability, and activation of fatty acid-sensing receptors could potentially contribute. Because the administered oils were not directly

analyzed and these mechanisms were not tested, these explanations remain speculative.

The dissociation between preload and postload conditions suggests that lipid-induced hormone secretion depends on nutrient sequence. When glucose is administered first, its rapid absorption may suppress α -cell responsiveness to fatty acids, thereby attenuating lipid-induced glucagon secretion (26–28); a preload may instead activate fatty acid sensing before substantial glycemic elevation (29–31). By contrast, the mechanisms underlying the stimulation of GLP-1 and GIP induced by OG remain uncertain. Early lipid delivery may facilitate activation of fatty acid-sensitive pathways in enteroendocrine K and L cells before the onset of glucose-induced changes in gastric emptying and intestinal transit, although in our study, the relative contributions of direct luminal sensing and secondary regulatory mechanisms could not be discriminated. Nevertheless, the concurrent augmentation of glucagon, GLP-1, and GIP suggests that OG elicits a coordinated foregut hormonal response that slows gastric emptying and attenuates postprandial glycemic excursions. Interestingly, the increase in the AUC-insulin/AUC-glucose ratio after OG occurred without a substantial increase in absolute insulin, consistent with maintained enteroinsular efficiency during reduced nutrient delivery, rather than enhanced intrinsic β -cell secretory capacity. Similarly, intravenous GLP-1 can improve glycemia while lowering postprandial insulin because slowing gastric emptying outweighs its insulinotropic effects (32).

GLP-1 is a physiological modulator of gastric emptying in humans (33) and acts through direct and vagally mediated mechanisms (33–35). Glucagon, traditionally viewed as a counterregulatory hormone, has also been shown to

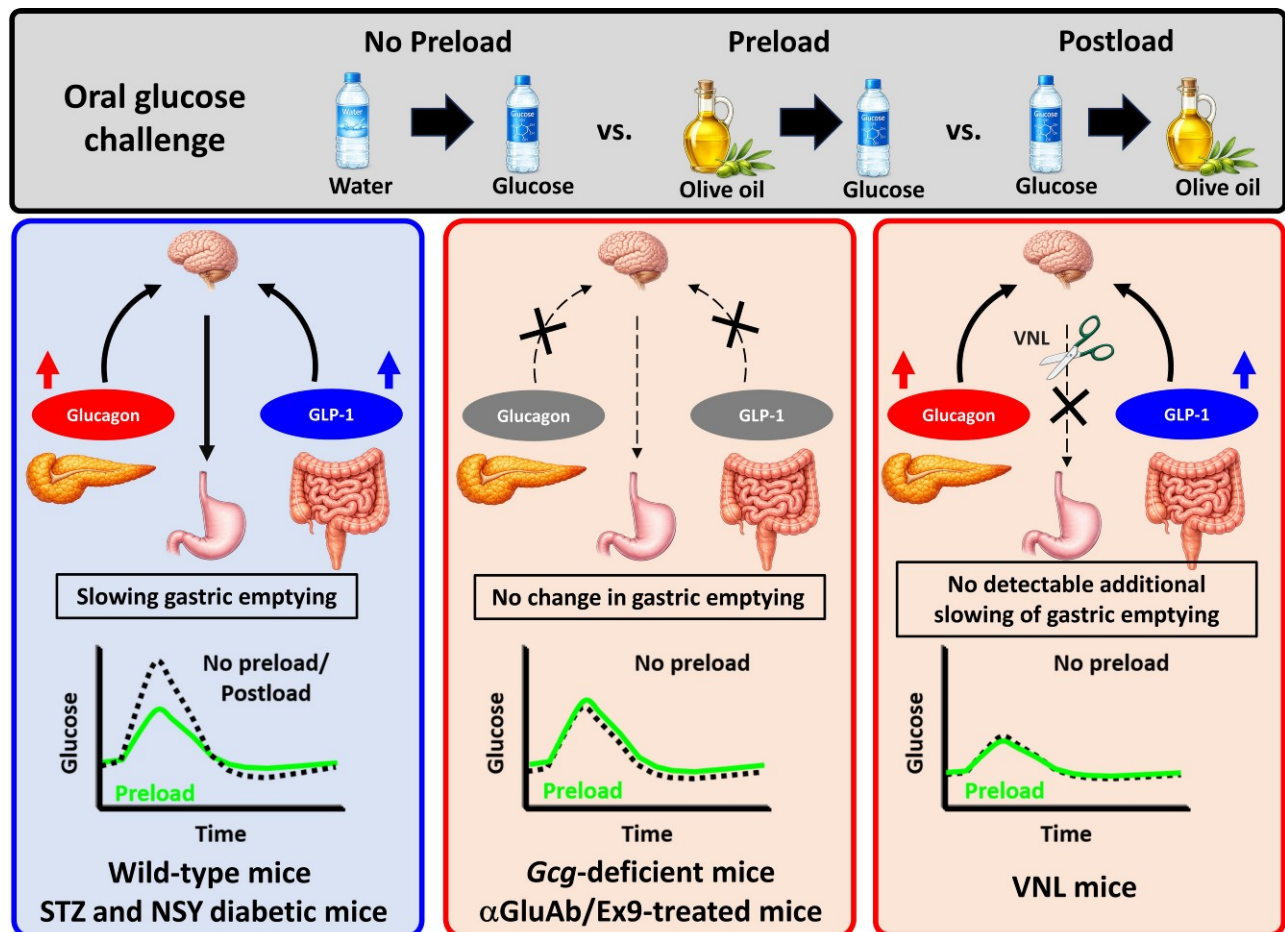


Figure 6—Proposed endocrine-neural mechanism underlying glucose lowering by OG. OG induces early secretion of glucagon and GLP-1, delays gastric emptying, and attenuates postchallenge glucose excursions in wild-type mice, as well as in STZ-induced insulin-deficient diabetic mice and NSY diabetic mice. Loss of the response in *Gcg*-deficient mice is indicative of a contribution of proglucagon-derived signaling, whereas the attenuation of glucose lowering and loss of detectable gastric slowing during combined glucagon receptor blockade and GLP-1 receptor antagonism support the concept of partially redundant contributions of glucagon and GLP-1 receptor signaling. In bilateral subdiaphragmatic VNL mice, hormone secretion is preserved or enhanced, whereas the effects of OG on glucose excursions and gastric emptying are attenuated. These findings support a contribution of vagal signaling to the coupling of endocrine responses with gastric motor regulation, although the VNL-induced delay in basal gastric emptying precludes concluding that intact vagal transmission is essential.

delay gastric emptying (36). Consistent with this concept, OG had no detectable effects on either glucose lowering or gastric emptying in *Gcg*-deficient mice, despite evidence that *Gcg* deficiency per se does not affect gastric emptying (37). In contrast, these effects were essentially preserved in *Glp1r*-deficient mice, indicating that proglucagon-derived peptides are a prerequisite for the phenotype, whereas GLP-1 receptor signaling alone is insufficient. Pharmacological studies further supported this relationship: Blockade of either receptor alone preserved the response, whereas combined blockade attenuated glucose lowering and eliminated detectable gastric slowing. These observations, accordingly, support the concept of partial redundancy between endogenous glucagon and GLP-1 receptor pathways. Although glucagon stimulates hepatic glucose production (38), reduced gastric nutrient delivery may outweigh this glucose-raising action under preload conditions (15,39). CCK-A receptor signaling was not indispensable because

OG remained effective in *Cckar*-deficient mice, although CCK may still have a modulatory role (40,41). Likewise, increased GIP secretion was consistently observed; however, previous studies suggested that endogenous GIP has little effect on gastric emptying in humans (42).

Bilateral subdiaphragmatic VNL per se affected the glucose excursion during OGTT, suggesting that basal vagal tone may contribute to systemic glucose homeostasis. However, the direction and magnitude of this effect may depend on the experimental context and involve complex regulation of insulin sensitivity and hepatic glucose production. Because previous studies have reported both insulin-sensitizing and insulin-opposing effects of vagal activation, the underlying mechanisms remain uncertain, and this issue did not represent a primary focus of the current study. Vagal function may also differ between basal and nutrient-stimulated conditions: Whereas basal vagal tone contributes to systemic metabolic regulation, nutrient-induced activation of vagal circuits

may have a distinct role in coordinating gastrointestinal motility and postprandial responses. After VNL, the glucose-lowering and gastric-emptying effects of OG were attenuated or no longer detected, whereas glucagon, GIP, and GLP-1 responses were preserved or enhanced. This dissociation supports a role for vagal pathways in coupling hormone signals to gastric motor function. The augmented hormone responses after VNL may reflect several nonmutually exclusive mechanisms, including altered gastrointestinal transit and prolonged intestinal nutrient exposure, loss of tonic vagal inhibition of enteroendocrine secretion, and adaptive neuroendocrine changes following vagal disruption. Because these mechanisms were not directly examined, the current study cannot discriminate among these possibilities. Together, these findings suggest that enteroendocrine hormone secretion can occur independently of intact vagal signaling, whereas vagal pathways may contribute to translating endocrine signals into coordinated gastric motor responses. This interpretation is consistent with the neuroincretin concept, which distinguishes hormone secretion from downstream physiological action. Both GLP-1 and glucagon have been shown to engage vagal afferent pathways and regulate gastric emptying (43–45). Taken together, our findings support a model in which OG activates an endocrine-vagal pathway that contributes to the downstream slowing of gastric emptying. However, because VNL itself markedly delayed gastric emptying, these findings do not establish that intact vagal transmission is essential.

The preservation of the effect of OG in diabetic models has potential clinical relevance. In STZ-induced diabetic mice, OG remained effective despite severely impaired insulin secretion, indicating that β -cell-derived hormones are unlikely to be the principal mediators of the acute response. Although amylin is cosecreted with insulin and may delay gastric emptying, the absence of an increased insulin response and the preserved effects of OG in severely insulin-deficient mice argue against increased insulin/amylin secretion as the principal mechanism. Given that gastric emptying is a major determinant of postprandial glycemia in both healthy individuals and in diabetes (14,15), interventions targeting slowing of gastric emptying may remain effective even when β -cell reserve is limited. In both humans who are healthy and humans with T2D, preloads of various fats have been shown to be effective in glucose lowering (46), but the current findings in mice require clinical testing.

Several limitations of our study should be appreciated. First, acetaminophen appearance reflects liquid-phase gastric emptying, is influenced by intestinal absorption and hepatic metabolism, and may correlate only weakly with scintigraphy, the gold standard method for assessing gastric emptying. Thus, confirmation using direct methodologies and solid mixed meals is warranted. Second, amylin was not measured, and a contributory role in the effects of OG, therefore, cannot be excluded. Third, although both pharmacological and genetic approaches support the role of glucagon, direct

evaluation using glucagon receptor-deficient models would further clarify receptor-specific contributions. Fourth, VNL itself slowed gastric emptying, and the 2-week interval may have been insufficient for complete physiological adaptation; altered basal motility and a ceiling effect may have obscured additional slowing by OG. Finally, 5 mL/kg olive oil represents a substantial acute fat load. Long-term metabolic effects, feasibility, and adherence with dietary-relevant doses in free-living humans remain to be determined.

In conclusion, OG reduced early glycemic excursions by slowing gastric emptying, with partially redundant contributions from glucagon and GLP-1 receptor signaling and involvement of vagal pathways. These findings provide a mechanistic basis for evaluating meal sequence strategies in both health and diabetes (Fig. 6).

Acknowledgments. The authors thank M. Yato and Y. Ogiso of Gifu University and Y. Tanaka, A. Yasui, and K. Hashimoto of Kyoto University for valuable secretarial support.

During the preparation of this work, the authors used ChatGPT Plus (OpenAI) for the purpose of language editing and improvement of readability. The authors take full responsibility for all the content of this article.

D.Y. is an editor of *Diabetes* but was not involved in any of the decisions regarding review of the manuscript or its acceptance.

Funding. This study was supported by KAKENHI from the Japan Society of Promotion of Science (to D.Y.), a research grant from the Japan Association for Diabetes Education and Care (to D.Y. and Y.L.), a research grant for specific diseases from the Suzuken Memorial Foundation (to D.Y.), and the Practical Research Project for Lifestyle-Related Diseases, including Cardiovascular Diseases and Diabetes Mellitus, funded by the Japan Agency for Medical Research and Development (to Yu.S.).

Duality of Interest. D.Y. received clinically commissioned/joint research grants from Novo Nordisk, Ono Pharmaceutical, Taisho Pharmaceutical, Terumo, and Arkray and consulting or speaker fees from Sumitomo Dainippon Pharma, Boehringer Ingelheim, Astellas Pharma, MSD, Novo Nordisk, Ono Pharmaceutical, Eli Lilly, and Takeda Pharmaceutical. T.K. received clinically commissioned/joint research grants from LifeScan and consulting or speaker fees from Novo Nordisk, Eli Lilly, and Tanabe Pharma Corporation. Y.Ho. received clinically commissioned/joint research grants from Boehringer Ingelheim and consulting or speaker fees from Sumitomo Pharma. S.T. received speaking and lecture fees from Mochida Pharmaceutical, Sanwa Kagaku Kenkyusho, Sumitomo Pharma, Daiichi Sankyo; Eli Lilly Japan, Mitsubishi Tanabe Pharma, Nippon Boehringer Ingelheim, AstraZeneca, Sanofi, Taisho Pharmaceutical, Abbott Japan, Novo Nordisk Pharma; Bayer Yakuhin, DexCom Japan, Teijin Pharma, and Amgen. Y.Yamaz. received speaker fees from Sumitomo Pharma, Novo Nordisk Pharma, Tanabe Pharma, Eli Lilly, Meiji, and Taisho Pharmaceutical. Y.Yamad. received speaker fees from Sumitomo Pharma, Novo Nordisk Pharma, Teijin Pharma, and Ono Pharmaceutical. H.F. received research grants from S&B Foods and speaker fees from Novo Nordisk, Sumitomo Pharma, and Daiichi Sankyo. A.S. received consulting or speaker fees from Amgen, Astellas Pharma, Daiichi Sankyo, Eli Lilly Japan, Kyowa Kirin, Novo Nordisk Pharma, Sumitomo Pharma, Taisho Pharmaceutical, Tanabe Pharma, and Teijin Pharma. D.J.D. is supported by a Banting and Best Diabetes Centre Novo Nordisk Chair in Incretin Biology, a Sinai Health–Novo Nordisk Foundation Fund in Regulatory Peptides, Canadian Institutes for Health Research grants 154321 and 192044, and Diabetes Canada–Canadian Cancer Society grant OG-3-24-5819-DD and has received consulting fees from Amgen, AstraZeneca, Alnylam, Crinetics Eli Lilly, General Medicines, Insulet, Kallyope, Metsara, Pfizer, and Protagonist Therapeutics and speaking fees from Novo Nordisk. Mt. Sinai Hospital has received investigator-initiated grant support from Amgen, Eli Lilly, and Zealand

Pharmaceuticals to support preclinical studies in the Drucker laboratory and received joint research grants from Sumitomo Pharma and Tanabe Pharma. M.H. was a member of the scientific and clinical advisory board of Glyscend Therapeutics, a company developing orally administered, gut-targeted polymer therapies. Glyscend Therapeutics ceased operations in January 2025, and any stock options became null and void. M.H. is an unpaid member of the clinical advisory boards of Alstemi Medical and Altrix Bio and reports travel support from the European Association for the Study of Diabetes. Yut.S. received clinically commissioned/joint research grants from Novo Nordisk Pharma, Taisho Pharmaceutical, Sumitomo Pharma, and Arkray Marketing and consulting or speaker fees from Sumitomo Pharma, Novo Nordisk Pharma, Tanabe Pharma, Eli Lilly, Nippon Becton Dickinson Company, and Taisho Pharmaceutical. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. Y.L., H.T., S.K., S.K.-O., H.K., Y.F., H.F., K.I., N.M., Yus.S., T.S., Y.I., Y.Se., Y.T., T.I., and D.Y. contributed to the data acquisition and interpretation. Y.L. and D.Y. contributed to the study concept and design and manuscript preparation. T.K., Y.Ho., S.T., Y.Yamaz., Y.Yamad., A.S., Y.Ha., D.J.D., Y.Sh., T.Y., T.M., M.H., and Yut.S. contributed to the discussion and review for intellectual content. All authors reviewed and approved the final version of the manuscript. D.Y. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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