

Long-Chain Free Fatty Acid Receptor GPR120 Mediates Oil-Induced GIP Secretion Through CCK in Male Mice

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Free fatty acid receptors GPR120 and GPR40 are involved in the secretion of gut hormones. GPR120 and GPR40 are expressed in enteroendocrine K cells, and their activation induces the secretion of the incretin glucose-dependent insulinotropic polypeptide (GIP). However, the role of these receptors in fat-induced GIP secretion *in vivo* and the associated mechanisms are unclear. In this study, we investigated corn oil-induced GIP secretion in GPR120-knockout (GPR120^{-/-}) and GPR40-knockout (GPR40^{-/-}) mice. Oil-induced GIP secretion was reduced by 50% and 80% in GPR120^{-/-} and GPR40^{-/-} mice, respectively, compared with wild-type mice. This was not associated with a significant difference in K-cell number or GIP content in K cells, nor messenger RNA levels of the lipid receptor GPR119, nor bile acid receptors TGR5 and farnesoid X receptor. GPR120^{-/-} and GPR40^{-/-} mice also exhibited substantially decreased levels of cholecystokinin (CCK), a hormone from I cells that promotes bile and pancreatic lipase secretion, and this decrease was associated with impaired gallbladder contraction. Notably, treatment with a CCK analog resulted in recovery of oil-induced GIP secretion in GPR120^{-/-} mice but not in GPR40^{-/-} mice. These results indicate that corn oil-induced GIP secretion from K cells involves both GPR120 and GPR40 signaling pathways, and GPR120-induced GIP secretion is indirectly mediated by CCK. (*Endocrinology* 158: 1172–1180, 2017)

Obesity is one of the most challenging public health problems confronting us and is a major risk factor for type 2 diabetes mellitus and cardiovascular disease (1). A reduction in obesity can contribute to the prevention and improvement of type 2 diabetes mellitus and arteriosclerosis. An inactive lifestyle and the consumption of high-calorie food and a high-fat diet promote obesity.

Gut hormones are secreted from enteroendocrine cells in a nutrient-modulated manner and play an important role in food intake, nutrient absorption, energy accumulation, and glucose homeostasis. For example, cholecystokinin (CCK) secreted from I cells induces bile and pancreatic lipase secretion (2). Secretin released

from S cells increases bicarbonate secretion for neutralization of gastric acid in duodenum (3). Ghrelin secreted from ϵ cells in the stomach increases food intake (4). Neurotensin (NTS) secreted from N cells promotes intestinal fat absorption (5). Gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide (GIP) and glucagonlike peptide 1 (GLP-1) are incretin hormones secreted from enteroendocrine K cells and L cells, respectively, that potentiate glucose-dependent insulin secretion from pancreatic β cells (6). GIP and GLP-1 also have extrapancreatic effects; for instance, pharmacological activation of the GLP-1 receptor reduces food intake through the central nervous system, whereas GIP directly increases glucose and triglyceride

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Abbreviations: AUC, area under the curve; CCK, cholecystokinin; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescence protein; GIP, gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide; GLP-1, glucagonlike peptide 1; GPR120^{-/-}, GPR120 knockout; GPR40^{-/-}, GPR40 knockout; LCFA, long-chain free fatty acid; mRNA, messenger RNA; NTS, neurotensin; OCTT, oral corn oil tolerance test; PCR, polymerase chain reaction; WT, wild-type.

uptake into adipocytes (7, 8). Peptide YY is coreleased from L cells with GLP-1 during meals and inhibits food intake (9).

Fat ingestion induces the secretion of CCK, GIP, and GLP-1. We previously reported that a reduction in GIP secretion alleviates high-fat-diet-induced obesity and insulin resistance (10). Thus, understanding the mechanisms of fat-induced GIP secretion may lead to a therapeutic strategy for obesity. Recently, GPR120 (free fatty acid receptor 4) and GPR40 (free fatty acid receptor 1) were identified as major long-chain free fatty acid (LCFA) receptors (11, 12). GPR120 is expressed in adipocytes and macrophages and is involved in the development of chronic inflammation and insulin resistance (13). High-fat-diet-fed GPR120-knockout (GPR120^{-/-}) mice gain more body weight and develop greater insulin resistance than high-fat-diet-fed wild-type (WT) mice (13, 14). GPR40 is highly expressed in β cells and is involved in insulin secretion in response to free fatty acids. GPR40-knockout (GPR40^{-/-}) mice are protected from obesity-induced hyperinsulinemia, hepatic steatosis, and hypertriglyceridemia (15). Interestingly, GPR120 and GPR40 were recently found to be expressed in enteroendocrine cells and involved in mediating incretin secretion (16, 17). For instance, both GPR120 and GPR40 are expressed in K cells and contribute to GIP secretion after ingestion of meals containing fat (16, 17). However, the relative contributions of GPR120 and GPR40 to fat-induced GIP secretion *in vivo* remain unclear. Also, the mechanism of fat-induced GIP secretion through GPR120 is not known, and this is particularly interesting, given that GPR120 is expressed in multiple enteroendocrine cell types.

In this study, we investigated GIP release in both GPR120^{-/-} mice and GPR40^{-/-} mice and established GPR120-dependent release of CCK as a mechanism of oil-induced GIP secretion.

Research Design and Methods

Animals

GPR120^{-/-} (14) and GPR40^{-/-} (18) mice were obtained to establish breeding colonies. For analysis of K-cell number and gene expression in K cells, GPR120^{-/-} and GPR40^{-/-} mice were crossbred with GIP-green fluorescence protein (GFP) knock-in (GIP-GFP) mice (19), which enabled us to visualize and isolate K cells by GFP, as described later. All mice used in this study were age-matched (9 to 16 weeks old) males maintained on the C57/BL6J background. All mice were housed in a temperature-controlled environment under conditions of a 14/10 light/dark cycle (14 hours of light and 10 hours of dark), with free access to water and food, unless otherwise stated. Animal care and protocols were all approved by the Animal Care Committee of Kyoto University (MedKyo15298).

Oral corn oil tolerance test

After a 16-hour fasting period, oral corn oil tolerance tests (OCTTs) (10 mL/kg body weight) were performed. Blood samples (45 μ L) were collected via tail vein at 0, 30, 60, and 120 minutes after oral oil administration by oral gavage, and blood glucose, plasma insulin, total GIP, and total GLP-1 levels were measured. Blood glucose levels were determined by the glucose oxidase method (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma insulin, total GIP, and total GLP-1 levels were measured by an insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Shibukawa, Japan), total GIP ELISA kit (Merck Millipore, Billerica, MA), and total GLP-1 ELISA kit (Meso Scale Discovery, Rockville, MD), respectively. For the measurements of CCK and NTS, we anesthetized mice with isoflurane inhalation and collected blood samples via portal vein 30 minutes after oral corn oil administration. CCK levels were measured in 300- μ L plasma samples using a radioimmunoassay kit (Euro Diagnostica, Malmö, Sweden). NTS levels were measured in 25- μ L plasma samples with an ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA). In the OCTT regimen, using a CCK analog, OCTTs were performed immediately after intravenous injection of [Thr28, Nle31]-CCK (#22944; AnaSpec, Silicon Valley, CA; 200 ng/kg body weight) (20) or phosphate-buffered saline via tail vein. Blood samples were collected 60 minutes after administration, and total GIP levels were measured.

Gallbladder contraction and bile volume after oral corn oil administration

The gallbladder volume was evaluated by ultrasonography 0 and 60 minutes after oral corn oil administration. The volume was calculated using the following equation: gallbladder volume (μ L) = length (mm) $\times$ width (mm) $\times$ depth (mm) $\times$ π / 6 (21). The amount of bile at 60 minutes after oral corn-oil administration was measured directly using a 29-gauge syringe (Terumo, Tokyo, Japan) immediately after cervical dislocation.

Isolation of K cells from mouse intestinal epithelium

The protocol to isolate K cells from murine intestinal epithelium was described previously (19). Briefly, the small intestine was divided in half, and the oral and rectal portions were defined as the upper and lower small intestine, respectively. The intestinal epithelium was digested with collagenase P (Roche Diagnostics, Basel, Switzerland) and collected into Dulbecco's modified Eagle medium (DMEM). After centrifugation at 180 g for 5 minutes, samples were resuspended with phosphate-buffered saline and filtered with a cell strainer (no. 352340; Falcon, Nagoya, Japan). The number of GFP-positive cells (K cells) in the intestinal epithelium was analyzed using a BD FACS Aria flow cytometer (Becton Dickinson, San Jose, CA). Sorted K cells were collected in vials containing extraction medium at a rate of 2000 cells per tube. Total RNA was extracted using the Picopure RNA isolation kit (Applied Biosystems, Alameda, CA) and treated with deoxyribonuclease (Qiagen, Valencia, CA). GIP content in isolated K cells (5000 cells per tube) was measured using the total GIP ELISA kit (Merck Millipore, Billerica, MA).

Quantitative real-time polymerase chain reaction

Complementary DNA was prepared by reverse transcription (Invitrogen, Carlsbad, CA) with an oligo-deoxythymidine primer (Invitrogen). The expression levels of messenger RNA (mRNA)

were measured by quantitative real-time polymerase chain reaction (PCR) using ABI PRISM 7000 Sequence Detection System (Applied Biosystems). SYBR Green PCR master mix (Applied Biosystems) was used for real-time PCR. PCR analyses were carried out using specific oligonucleotide primers. All results are presented using the ΔC_t method normalized to a reference gene (β -actin). Primer sequences were as follows: β -actin, 5'-TGTTACCAACTGGGACGAC-3' and 5'-GGGGTGTGAAGGTCTCAA-3'; GPR40, 5'-TTTGCGCTGGGCTTTCC-3' and 5'-GCTGGGAGTGAGTGAGTCGCAGTT-3'; TGR5, 5'-GCCTCTGTCAGTCTTGGCCTAT-3' and 5'-TTCCTCGAAGCACTCGTAGACA-3'; farnesoid X receptor, 5'-CCTGAGAA-CCCACAGCATTT-3' and 5'-GTGTCCATCACTGCACATCC-3'; GLP-1, 5'-TGAAGACAAACGCCAC-3' and 5'-TCATGACGTTTGGCAA-3'; CCK, 5'-CGCTGGAAGTGCCTGAC-3' and 5'-GCGGCCAGAAAGGAGCTTTGC-3'; secretin, 5'-TGGATGGGTCCCTGTCTCTCO-3' and 5'-TGATGCCAAGGACAACCAAT-3'; and NTS, 5'-CTGGTGTGCCTGACTCTCT-3' and 5'-TCACATCTTCTTCTGAATCTGAGC-3'. Others were previously described (16).

Statistical analysis

All data are presented as mean $\pm$ standard error of the mean. Statistical analyses were performed using one-way analysis of variance with Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant.

Results

GPR120 and GPR40 play an important role in oil-induced GIP secretion

OCTTs were performed on WT, GPR120^{-/-}, and GPR40^{-/-} mice. Blood glucose and plasma insulin levels in GPR120^{-/-} mice were similar to those in WT mice [Fig. 1(a) and 1(b)]. Blood glucose levels at 0, 60, and 120 minutes during the OCTT and area under the curve (AUC) were significantly higher in GPR40^{-/-} mice than in WT mice [Fig. 1(a)]. Insulin levels tended to be lower in GPR40^{-/-} mice than in WT mice, and AUC insulin was significantly lower in the GPR40^{-/-} mice compared with GPR120^{-/-} mice. Plasma total GIP levels were significantly lower after corn oil ingestion in both GPR120^{-/-} and GPR40^{-/-} mice compared with WT mice; levels in GPR40^{-/-} mice were the lowest [Fig. 1(c)]. AUC GIP was reduced in GPR120^{-/-} mice by 50% compared with that in WT mice, whereas AUC GIP was reduced in GPR40^{-/-} mice by 80% compared with that in WT mice. In contrast, there was no significant difference in total GLP-1 levels and AUC GLP-1 among the three groups [Fig. 1(d)]. Intestinal transit estimated using oil red O (22) was not significantly different among the three groups (data not shown).

CCK secretion and gallbladder contraction are impaired in GPR120^{-/-} mice, resulting in reduced GIP secretion after oil ingestion

In preliminary studies, we determined that CCK levels were undetectable at 0 and 60 minutes during OCTTs in

WT mice, with peak levels observed at 30 minutes (data not shown). Subsequently, CCK levels were measured at 30 minutes during OCTTs in WT, GPR120^{-/-}, and GPR40^{-/-} mice [Fig. 2(a)]. CCK levels were significantly lower in GPR120^{-/-} and GPR40^{-/-} mice than in WT mice, but there was no significant difference in the levels between GPR120^{-/-} and GPR40^{-/-} mice. Gallbladder volume was measured before or after corn oil ingestion using ultrasonography, because CCK is known to induce gallbladder contractions [Fig. 2(b)]. Gallbladder volume after corn oil ingestion was significantly decreased in WT mice, but the contraction was inhibited in GPR120^{-/-} and GPR40^{-/-} mice [Fig. 2(b)]. Gallbladders contracted by 71.0% $\pm$ 1.3%, 31.5% $\pm$ 9.5%, and 6.4% $\pm$ 5.6%, in WT, GPR120^{-/-}, and GPR40^{-/-} mice, respectively. Direct measurement of bile volume indicated that, among the three groups, the volume after corn oil ingestion was the highest in GPR40^{-/-} mice [Fig. 2(c)]. Intravenous administration of CCK analog (200 ng/kg body weight) resulted in complete contraction of gallbladders in WT, GPR120^{-/-}, and GPR40^{-/-} mice (data not shown). These results indicate that GPR120^{-/-} and GPR40^{-/-} mice have impaired gallbladder contraction after oil ingestion, likely because of reduced CCK secretion.

Corn oil ingestion significantly increased GIP levels at 60 minutes in WT mice similarly in the presence or absence of CCK analog treatment [Fig. 2(d)]. In contrast, GIP levels were only modestly increased after corn oil ingestion in GPR120^{-/-} and GPR40^{-/-} mice in the absence of CCK analog treatment, whereas treatment with the CCK analog restored GIP responses in GPR120^{-/-} mice but not in GPR40^{-/-} mice [Fig. 2(d)]. Fasting GIP levels were not significantly different between WT, GPR120^{-/-}, and GPR40^{-/-} mice.

GPR120 and GPR40 are expressed in K cells, but K-cell number and GIP content are not altered in GPR120^{-/-} and GPR40^{-/-} mice

GPR120^{-/-} and GPR40^{-/-} mice were crossbred with GIP-GFP mice, and the resulting GIP-GFP heterozygous mice with the GPR120- or GPR40-knockout background were used to evaluate K-cell number and levels of selected genes in K cells. GPR120 and GPR40 were expressed in K cells of both the upper and lower small intestine in WT mice [Fig. 3(a) and 3(b)]. In K cells of GPR120^{-/-} mice, GPR120 mRNA was undetectable [Fig. 3(a)], whereas GPR40 mRNA levels were similar to levels in WT mice [Fig. 3(b)]. In K cells of GPR40^{-/-} mice, the expression levels of GPR120 mRNA were similar to those of WT mice [Fig. 3(a)], whereas GPR40 mRNA levels were undetectable [Fig. 3(b)]. These results indicate that there were no compensatory changes in the expression of these LCFA receptors in K cells of the knockout mice.

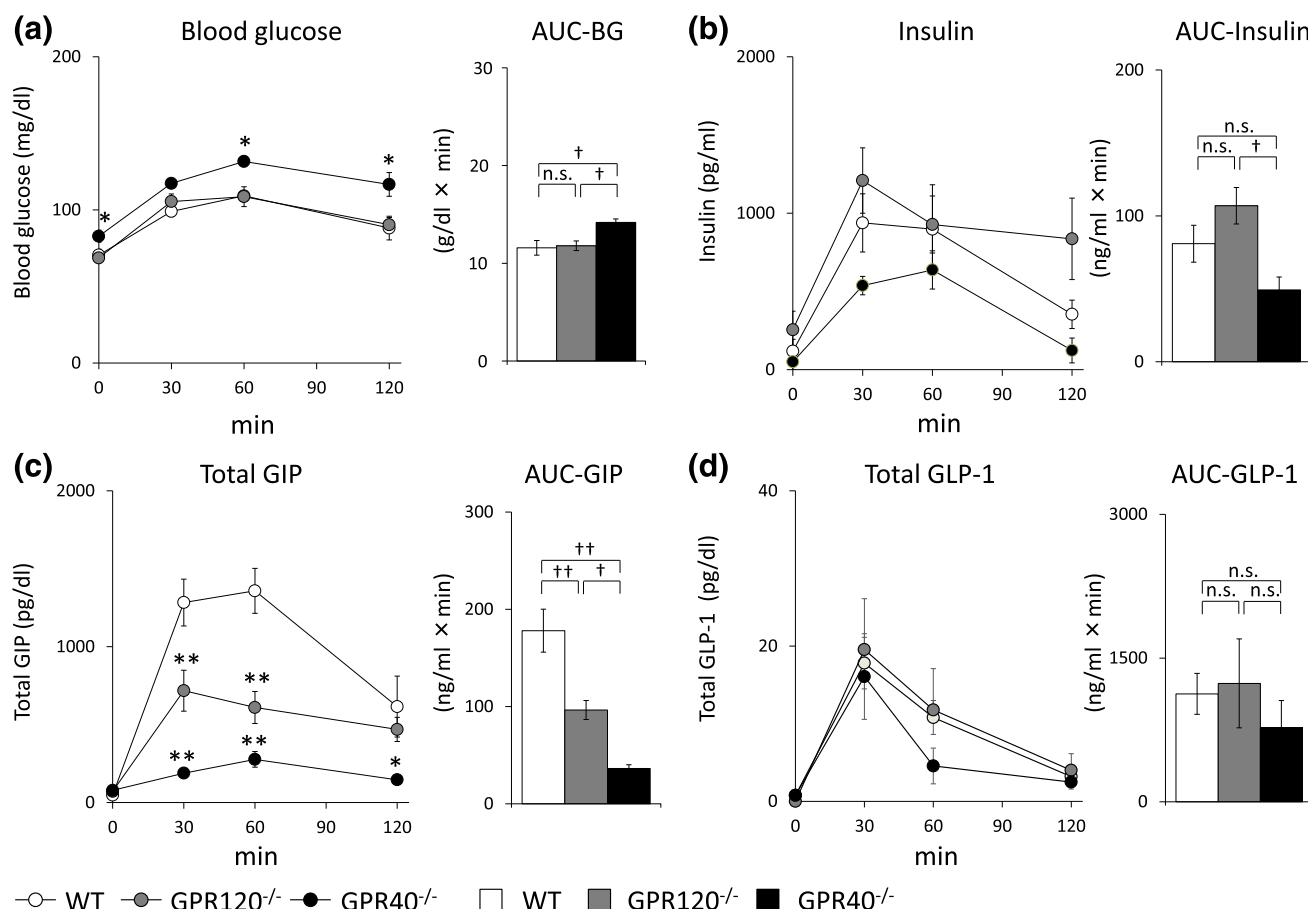


Figure 1. Blood glucose, insulin, and incretin levels during OCTT in GPR120^{-/-} and GPR40^{-/-} mice. (a) Blood glucose (BG), (b) insulin, (c) total GIP, and (d) total GLP-1 levels during OCTT (corn oil, 10 mL/kg) (n = 6). WT mice are represented by white circles and bars. GPR120^{-/-} mice are represented by gray circles and bars. GPR40^{-/-} mice are represented by black circles and bars. **P* < 0.05, ***P* < 0.01 vs WT mice. +*P* < 0.05, ++*P* < 0.01. n.s., no significance.

There was no significant difference in K-cell number in the upper and lower small intestine among the three groups [Fig. 3(c)]. Furthermore, GIP content and GIP mRNA expression in K cells were not significantly different among the three groups [Fig. 3(d) and 3(e)]. Moreover, GLP-1, CCK, and secretin mRNA expression levels were comparable between the three groups (Supplemental Fig. 1). There was a small increase in NTS expression in K cells isolated from GPR40^{-/-} mice compared with those from GPR120^{-/-} mice, but this was not reflected in differences in plasma levels of NTS 30 minutes after oral corn oil administration (WT mice, 0.82 ± 0.08 ng/mL; GPR120^{-/-} mice, 1.07 ± 0.11 ng/mL; and GPR40^{-/-} mice, 0.74 ± 0.09 ng/mL; n = 6, respectively). Thus, the absence of GPR120 or GPR40 does not affect the K-cell number and expression of GIP or other selected enteroendocrine hormones in K cells of the upper and lower intestine.

GPR120 and GPR40 do not affect GPR119 expression and bile-associated genes in K cells

GPR119 is a G protein-coupled receptor activated by fatty acid ethanolamides and is reported to be involved in

incretin secretion (23, 24). There were no significant differences in the expression levels of GPR119 mRNA in K cells of the upper and lower small intestine among the three groups [Fig. 4(a)]. Bile-associated genes were assessed in K cells, because gallbladder contraction was impaired in GPR120^{-/-} and GPR40^{-/-} mice after corn oil ingestion [Fig. 2(b) and 2(c)]. TGR5, a membrane-type receptor for bile acids, is reportedly associated with GLP-1 secretion (25). There was no significant difference in the expression level of TGR5 mRNA among the three groups [Fig. 4(b)]. Farnesoid X receptor is a nuclear receptor involved in the enterohepatic circulation of bile acids and incretin secretion (26), but its expression level in K cells was also not different among the three groups [Fig. 4(c)].

Discussion

In this study, we investigated the effect of GPR120 and GPR40 on corn oil-induced GIP secretion using GPR120^{-/-} and GPR40^{-/-} mice, and we observed a 50% and 80% reduction in GIP secretion compared with WT mice, respectively. Thus, GPR120 and GPR40 are associated with oil-induced GIP secretion in mice,

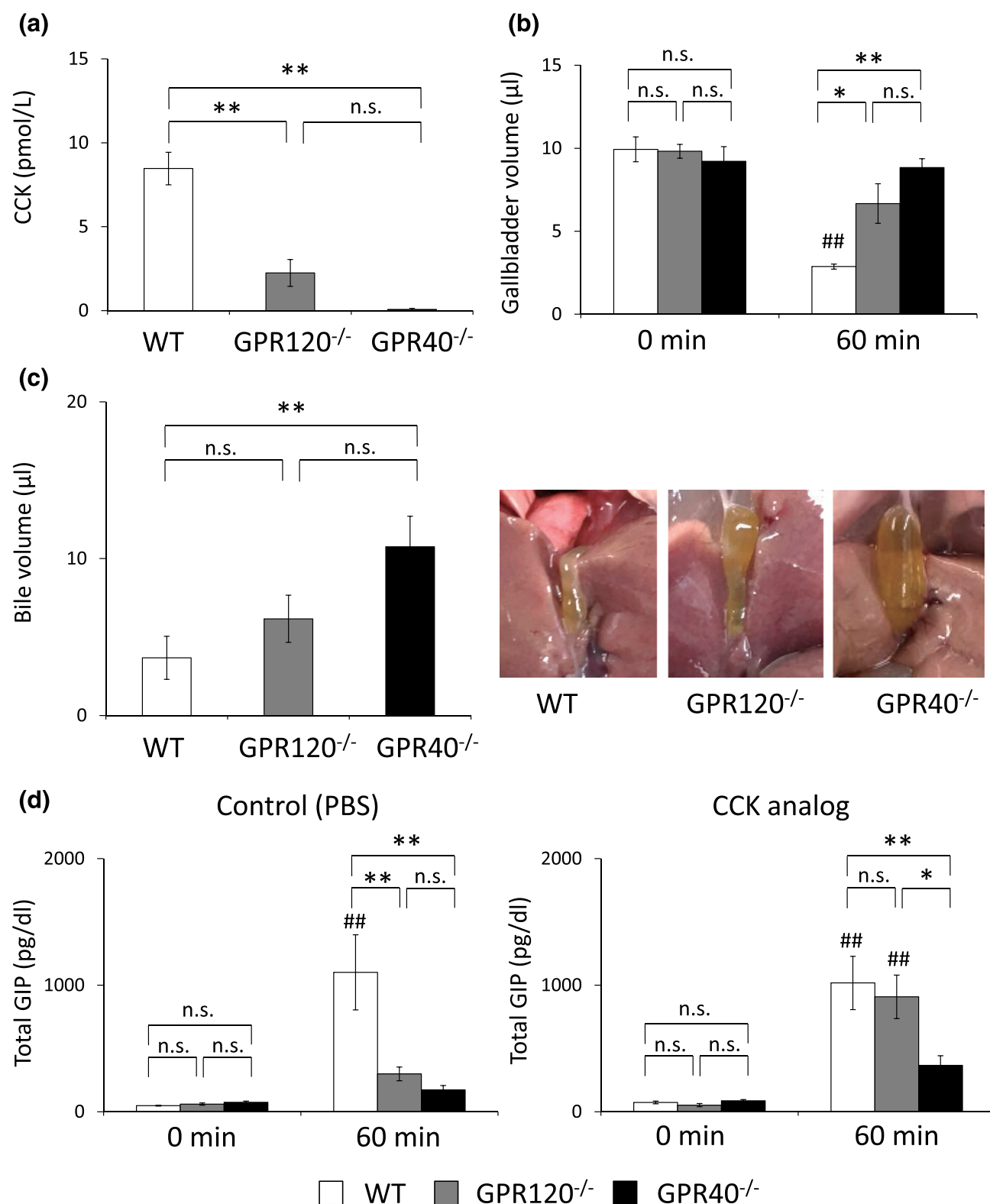


Figure 2. Effect of GPR120 and GPR40 on CCK levels and gallbladder contraction during OCTT. (a) CCK levels in portal vein 30 minutes after oral corn oil administration (10 mL/kg) ($n = 4$ to 7). (b) Gallbladder volume measured by ultrasonography at 0 and 60 minutes after corn oil administration ($n = 3$). (c) Direct measurement of bile volume remaining in gallbladder 60 minutes after oil administration ($n = 6$). (d) Effect of CCK analog on corn oil-induced GIP levels ($n = 6$ to 7). WT, GPR120^{-/-}, and GPR40^{-/-} mice are represented by white, gray, and black bars, respectively. * $P < 0.05$, ** $P < 0.01$ vs WT mice. ## $P < 0.01$ vs 0 minutes. n.s., no significance.

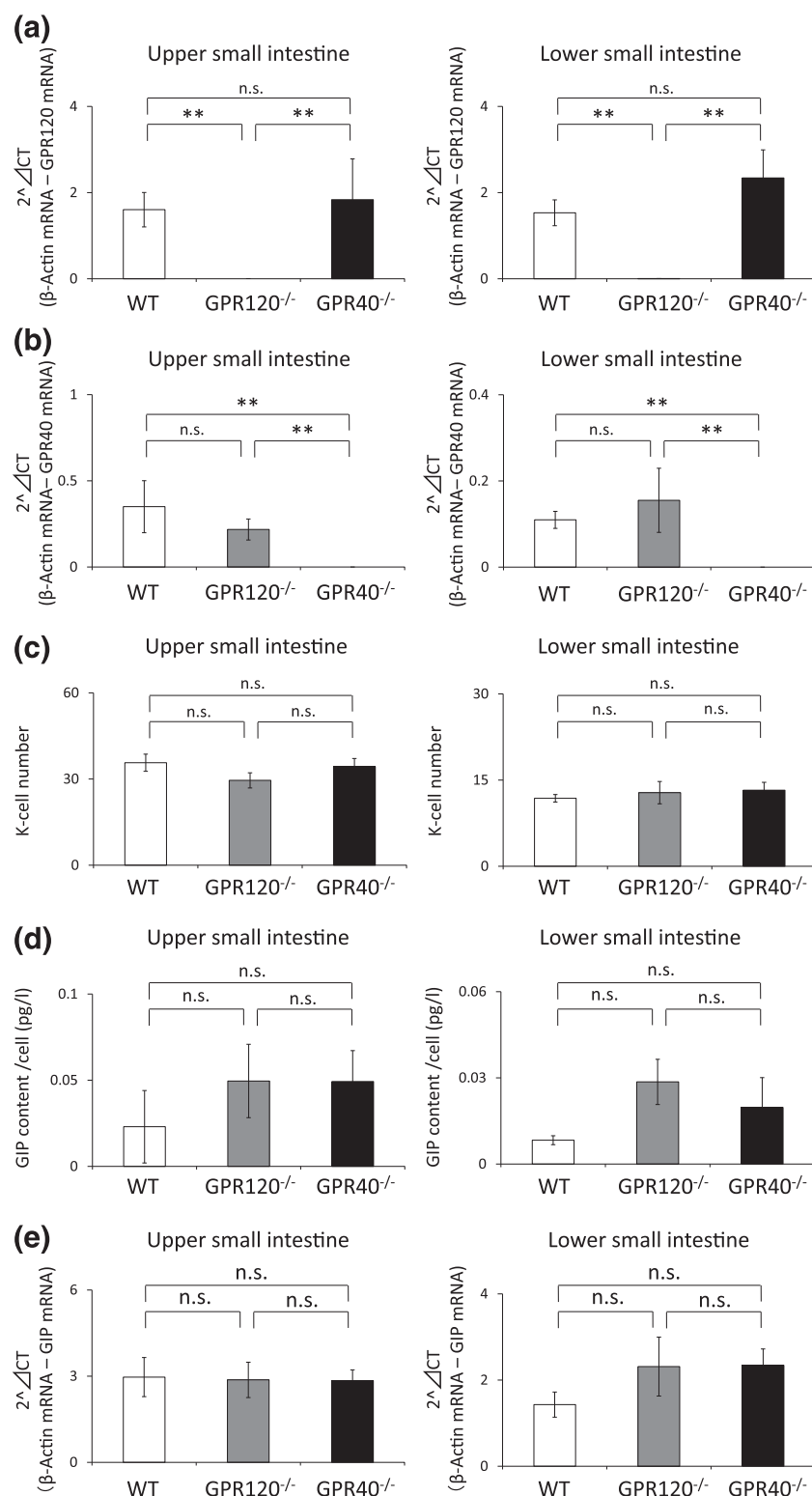


Figure 3. K-cell number and GIP expression in K cells in GPR120^{-/-} and GPR40^{-/-} mice. The expression levels of (a) GPR120 mRNA and (b) GPR40 mRNA in the K cells of WT, GPR120^{-/-}, and GPR40^{-/-} mice (n = 5 to 6). (c) K-cell number in WT, GPR120^{-/-}, and GPR40^{-/-} mice (n = 7 to 9). (d) GIP content and (e) GIP mRNA expression in K cells of WT, GPR120^{-/-}, and GPR40^{-/-} mice (n = 5 to 6). WT, GPR120^{-/-}, and GPR40^{-/-} mice are represented by white, gray, and black bars, respectively. **P < 0.01 vs WT mice. n.s., no significance.

although GPR40 was found to have the larger role. Ekberg *et al.* (27) recently reported reduced GIP levels after olive oil ingestion in GPR40^{-/-} mice but not in GPR120^{-/-} mice when compared with WT mice. Our results are compatible with their findings that GPR40 plays a greater role in oil-induced GIP secretion than does GPR120, and oil-induced GLP-1 secretion is not altered in either GPR120^{-/-} or GPR40^{-/-} mice. Although Ekberg *et al.* (27) concluded that GPR120 plays a minor role, if any, in oil-induced GIP secretion, we observed an important contribution of GPR120 in both our previous report (16) and the current study. Differences may be attributed to the fatty acid composition of the test oils and time points for measurement of plasma GIP levels. Oleic acid is the predominant fatty acid in the olive oil used by Ekberg *et al.* (27), whereas linoleic acid is the primary fatty acid in the corn oil we used (28). GIP levels were only measured at a single postprandial time (60 minutes) after olive oil ingestion in the study by Ekberg *et al.* (27), whereas we measured GIP levels at 30, 60, and 120 minutes after corn oil ingestion.

GPR120 and GPR40 are also reported to be associated with GLP-1 secretion. α -linolenic acid enhances GLP-1 secretion through GPR120 in mouse L cells (11). Moreover, postprandial GLP-1 levels are reduced in GPR40-knockout mice (17). However, in our studies, we did not observe decreased GLP-1 secretion in GPR120^{-/-} or GPR40^{-/-} mice. These results indicate that corn oil may not stimulate GLP-1 secretion as strongly as it stimulates GIP secretion through GPR120 and GPR40.

Although oil-induced GIP secretion was dramatically impaired in GPR120^{-/-} and GPR40^{-/-} mice, intestinal K-cell number and GIP content appeared normal. In addition, we determined that there is no reciprocal compensation for loss of GPR120 or GPR40 in K cells, nor altered expression of the ethanolamide receptor

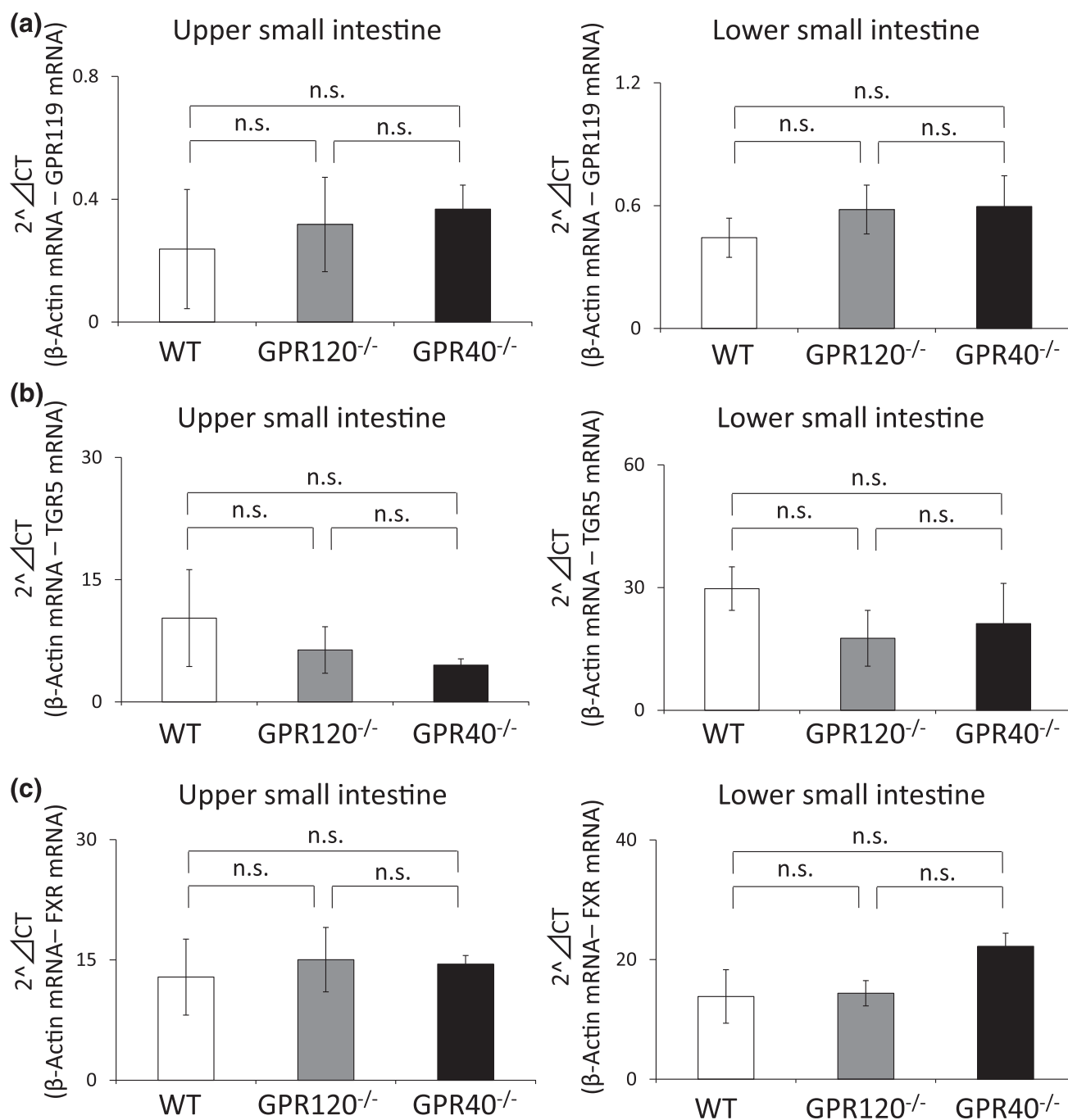


Figure 4. Expression of GPR119 and bile-associated genes in K cells in GPR120^{-/-} and GPR40^{-/-} mice. The mRNA expression levels of (a) GPR119, (b) TGR5, and (c) farnesoid X receptor (FXR) in K cells of WT, GPR120^{-/-}, and GPR40^{-/-} mice (n = 5 to 6). WT, GPR120^{-/-}, and GPR40^{-/-} mice are represented by white, gray, and black bars, respectively. n.s., no significance.

GPR119 or the bile acid receptor TGR5, which are involved in incretin secretion (16, 17, 23–25, 27). We concluded that the smaller effect on GIP secretion of loss of GPR120 versus loss of GPR40 cannot be attributed to changes in K-cell number nor to the expression levels of GIP or other candidate genes that are associated with incretin secretion.

We also evaluated mRNA expression levels of GLP-1, CCK, secretin, and NTS in K cells from WT, GPR120^{-/-},

and GPR40^{-/-} mice. Levels of NTS mRNA were elevated in K cells of the lower small intestine of GPR40^{-/-} mice relative to GPR120^{-/-} mice. Because NTS is reported to play a key role in bile reabsorption (29, 30), the upregulation of NTS mRNA expression in K cells in GPR40^{-/-} mice might be a compensatory response to the severe impairment of bile secretion in these animals. However, the circulating NTS levels were comparable among the three groups of mice.

GPR120 and GPR40 are expressed in I cells and are involved with CCK secretion (31, 32). CCK has an important role in promoting secretion of bile and pancreatic lipase. Lipase inhibitors decrease incretin secretion by decreasing the conversion of triglycerides to monoglycerides and fatty acids (33). We previously determined that bile is essential for GIP secretion after lard-oil ingestion using bile duct-ligated mice (22). To our knowledge, CCK secretion has not been compared between GPR120^{-/-} and GPR40^{-/-} mice. In the current study, we evaluated CCK levels and gallbladder contraction in these animals and found that CCK levels and gallbladder contraction after corn oil ingestion were significantly decreased in GPR120^{-/-} and GPR40^{-/-} mice compared with WT mice. These results raise the possibility that the impaired GIP secretion after oil ingestion in GPR120^{-/-} and GPR40^{-/-} mice could be mediated in part by reduced CCK secretion. To test this hypothesis, we treated the animals with a CCK analog that induced complete gallbladder contraction and observed fully recovered GIP levels at 60 minutes after corn oil ingestion in GPR120^{-/-} mice, to levels similar to those seen in WT mice, but GPR40^{-/-} mice did not. These results suggest that CCK secretion is the primary mediator of corn oil-induced GIP secretion through GPR120. In contrast, corn oil-induced GIP secretion mediated by GPR40 likely involves both direct effects on K cells and indirect effects mediated by CCK secretion. This dual mechanism of GPR40 on GIP secretion may explain our observation that GPR40 is a stronger mediator of corn oil-induced GIP secretion than GPR120. Although our own results and those of others (16, 34) indicate that GPR120 and GPR40 are highly expressed in K cells, it remains unclear to what extent they contribute directly to LCFA-induced GIP secretion, because we used mice with full-body knockout of these receptors. Further studies using mice with K-cell-specific GPR120 knockout and GPR40 knockout are warranted to elucidate the direct effect of GPR120 and GPR40 on LCFA-induced GIP secretion.

In conclusion, GPR120 contributes to oil-induced GIP secretion in mice by enhancing CCK secretion, whereas GPR40 contributes more substantially and is involved in both CCK-dependent and -independent GIP secretion *in vivo*. It is possible that the reduced GIP secretion in GPR40^{-/-} mice in part explains why such animals are protected from high-fat-diet-induced obesity (15). Assuming the same receptors are involved in promoting GIP secretion in humans, blocking these pathways could be a strategy to diminish weight gain.

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Author contributions: A.S. planned the study, researched data, contributed to discussions, and wrote, reviewed, and edited the manuscript. N.H. planned the study, contributed to discussions, and reviewed and edited the manuscript. K.I., S.Y., Y.M., K. Shibue, Y.T., and K. Suzuki researched data and contributed to discussions. T.H., Y.K., and S.S.-K. contributed to research of data. A.H. contributed to discussions. N.I. contributed to discussions and reviewed and edited the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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References

1. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metab*. 2011;14(5):575–585.
2. Liddle RA, Goldfine ID, Rosen MS, Taplitz RA, Williams JA. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J Clin Invest*. 1985;75(4):1144–1152.
3. Alpini G, Lenzi R, Sarkozi L, Tavoloni N. Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. *J Clin Invest*. 1988;81(2):569–578.
4. Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC. Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology*. 2006;147(1):23–30.
5. Li J, Song J, Zaytseva YY, Liu Y, Rychahou P, Jiang K, Starr ME, Kim JT, Harris JW, Yiannikouris FB, Katz WS, Nilsson PM, Orholm-Melander M, Chen J, Zhu H, Fahrenholz T, Higashi RM, Gao T, Morris AJ, Cassis LA, Fan TW, Weiss HL, Dobner PR, Melander O, Jia J, Evers BM. An obligatory role for neurotensin in high-fat-diet-induced obesity. *Nature*. 2016;533(7603):411–415.
6. Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, Hansotia T, Drucker DJ, Wollheim C, Burcelin R, Thorens B. Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest*. 2004;113(4):635–645.

7. Drucker DJ. The biology of incretin hormones. *Cell Metab.* 2006; 3(3):153–165.
8. Seino Y, Yabe D. Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: Incretin actions beyond the pancreas. *J Diabetes Investig.* 2013;4(2):108–130.
9. De Silva A, Salem V, Long CJ, Makwana A, Newbould RD, Rabiner EA, Ghatei MA, Bloom SR, Matthews PM, Beaver JD, Dhillon WS. The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metab.* 2011;14(5):700–706.
10. Nasteska D, Harada N, Suzuki K, Yamane S, Hamasaki A, Joo E, Iwasaki K, Shibue K, Harada T, Inagaki N. Chronic reduction of GIP secretion alleviates obesity and insulin resistance under high-fat diet conditions. *Diabetes.* 2014;63(7):2332–2343.
11. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med.* 2005;11(1):90–94.
12. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature.* 2003;422(6928):173–176.
13. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* 2010;142(5):687–698.
14. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoer C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffei C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Körner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Lévy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, Froguel P. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature.* 2012;483(7389):350–354.
15. Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H. The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab.* 2005;1(4):245–258.
16. Iwasaki K, Harada N, Sasaki K, Yamane S, Iida K, Suzuki K, Hamasaki A, Nasteska D, Shibue K, Joo E, Harada T, Hashimoto T, Asakawa Y, Hirasawa A, Inagaki N. Free fatty acid receptor GPR120 is highly expressed in enteroendocrine K cells of the upper small intestine and has a critical role in GIP secretion after fat ingestion. *Endocrinology.* 2015;156(3):837–846.
17. Edfalk S, Steneberg P, Edlund H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes.* 2008;57(9):2280–2287.
18. Matsuda-Nagasumi K, Takami-Esaki R, Iwachidow K, Yasuhara Y, Tanaka H, Ogi K, Nakata M, Yano T, Hinuma S, Taketomi S, Odaka H, Kaisho Y. Lack of GPR40/FFAR1 does not induce diabetes even under insulin resistance condition. *Diabetes Obes Metab.* 2013;15(6):538–545.
19. Suzuki K, Harada N, Yamane S, Nakamura Y, Sasaki K, Nasteska D, Joo E, Shibue K, Harada T, Hamasaki A, Toyoda K, Nagashima K, Inagaki N. Transcriptional regulatory factor X6 (Rfx6) increases gastric inhibitory polypeptide (GIP) expression in enteroendocrine K-cells and is involved in GIP hypersecretion in high fat diet-induced obesity. *J Biol Chem.* 2013;288(3):1929–1938.
20. Zhou L, Yang H, Okoro EU, Guo Z. Up-regulation of cholesterol absorption is a mechanism for cholecystokinin-induced hypercholesterolemia. *J Biol Chem.* 2014;289(19):12989–12999.
21. Everson GT, Braverman DZ, Johnson ML, Kern F Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder volume and contraction. *Gastroenterology.* 1980;79(1):40–46.
22. Shibue K, Yamane S, Harada N, Hamasaki A, Suzuki K, Joo E, Iwasaki K, Nasteska D, Harada T, Hayashi Y, Adachi Y, Owada Y, Takayanagi R, Inagaki N. Fatty acid-binding protein 5 regulates diet-induced obesity via GIP secretion from enteroendocrine K cells in response to fat ingestion. *Am J Physiol Endocrinol Metab.* 2015; 308(7):E583–E591.
23. Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, Chen R, Jones RM, Behan DP, Leonard J. A role for intestinal endocrine cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. *Endocrinology.* 2008;149(5):2038–2047.
24. Lauffer LM, Iakubov R, Brubaker PL. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. *Diabetes.* 2009;58(5): 1058–1066.
25. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Matak C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* 2009;10(3):167–177.
26. Trabelsi MS, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, Perino A, Brighton CA, Sebt Y, Kluza J, Briand O, Dehondt H, Vallez E, Dorchie E, Baud G, Spinelli V, Hennuyer N, Caron S, Bantubungi K, Caiazzo R, Reimann F, Marchetti P, Lefebvre P, Bäckhed F, Gribble FM, Schoonjans K, Pattou F, Talleux A, Staels B, Lestavel S. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun.* 2015;6: 7629.
27. Ekberg JH, Hauge M, Kristensen LV, Madsen AN, Engelstoft MS, Husted AS, Sichlau R, Egerod KL, Timshel P, Kowalski TJ, Gribble FM, Reiman F, Hansen HS, Howard AD, Holst B, Schwartz TW. GPR119, a major enteroendocrine sensor of dietary triglyceride metabolites coacting in synergy with FFA1 (GPR40). *Endocrinology.* 2016;157(12):4561–4569.
28. Maki KC, Lawless AL, Kelley KM, Kaden VN, Geiger CJ, Dicklin MR. Corn oil improves the plasma lipoprotein lipid profile compared with extra-virgin olive oil consumption in men and women with elevated cholesterol: results from a randomized controlled feeding trial. *J Clin Lipidol.* 2015;9(1):49–57.
29. Gui X, Dobner PR, Carraway RE. Endogenous neurotensin facilitates enterohepatic bile acid circulation by enhancing intestinal uptake in rats. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281(6):G1413–G1422.
30. Gui X, Carraway RE. Enhancement of jejunal absorption of conjugated bile acid by neurotensin in rats. *Gastroenterology.* 2001;120(1):151–160.
31. Liou AP, Lu X, Sei Y, Zhao X, Pechhold S, Carrero RJ, Raybould HE, Wank S. The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. *Gastroenterology.* 2011;140(3):903–912.
32. Tanaka T, Katsuma S, Adachi T, Koshimizu TA, Hirasawa A, Tsujimoto G. Free fatty acids induce cholecystokinin secretion through GPR120. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2008; 377(4–6):523–527.
33. Damci T, Yalin S, Balci H, Osar Z, Korugan U, Ozyazar M, Ilkova H. Orlistat augments postprandial increases in glucagon-like peptide 1 in obese type 2 diabetic patients. *Diabetes Care.* 2004; 27(5):1077–1080.
34. Parker HE, Habib AM, Rogers GJ, Gribble FM, Reimann F. Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia.* 2009; 52(2):289–298.