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TITLE

Novel mechanism of fatty acid sensing in enteroendocrine cells: Specific structures in oxo-fatty acids produced by gut bacteria are responsible for CCK secretion in STC-1 cells via GPR40

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Key words

Fatty acids; Gut lactic acid bacteria metabolites; Enteroendocrine cells; Cholecystokinin; Gastric emptying;

Abbreviations

CCK, cholecystokinin; C18, octadecanoic fatty acid; GPR40, G-protein coupled receptor 40; GPR120, G-protein coupled receptor 120; LDH, lactate dehydrogenase;

ABSTRACT

Scope

The secretion of gut hormones, such as cholecystokinin (CCK) is stimulated by fatty acids. Although a chain length-dependent mechanism has been proposed, other structural relationships to releasing activity remain unclear. We aimed to elucidate specific structures in fatty acids that are responsible for their CCK-releasing activity, and related sensing mechanisms in enteroendocrine cells.

Methods and results

We examined CCK secretory activities in a murine CCK-producing cell line STC-1 by exposing the cells to various modified fatty acids produced by gut lactic acid bacteria. The effects of fatty acids on gastric emptying rate as a CCK-mediated function were examined using acetaminophen- and phenol red-methods in rats. Out of more than thirty octadecanoic (C18)-derived fatty acids tested, five oxo-fatty acids potently stimulated CCK secretion without cytotoxic effects in STC-1 cells. Three fatty acids had a distinct specific structure containing one double-bond, whereas the other two had two double-bonds, nearby an oxo residue. CCK secretion induced by representative fatty acids (10-oxo-*trans*-11-18:1 and 13-oxo-*cis*-9,*cis*-15-18:2) was attenuated by a fatty acid-receptor GPR40 antagonist. Oral administration of 13-oxo-*cis*-9,*cis*-15-18:2 lowered the gastric emptying rate in rats in a dose- and structure-dependent manner.

Conclusion

These results revealed a novel fatty acid-sensing mechanism in enteroendocrine cells.

INTRODUCTION

Enteroendocrine systems have a critical role in maintaining whole body homeostasis, including gastrointestinal digestive and absorptive functions, nutrient metabolism, and feeding behavior in both pre- and postprandial states, through regulation of gut hormone secretions [1, 2]. Enteroendocrine cells sense luminal nutrients and then release a specific gut hormone. Macronutrients are potent stimulants for secretion of various gut hormones.

Cholecystokinin (CCK) is a gut hormone produced in enteroendocrine I cells, which are mainly located in the proximal small intestine [3, 4]. CCK has multiple physiological actions related to postprandial responses, such as induction of pancreatic enzyme secretion, and suppression of gastric emptying rate and appetite. Secretion of CCK is potently stimulated by luminal fatty acids and peptides. Dietary lipids (triglycerides) mainly consist of long chain fatty acids of more than 16- carbon chain length. Dietary fatty acids have a variety of structures based on their chain length, degree of unsaturation, position of double bond, and so on. The relationship between fatty acid structure and fatty acid-induced CCK secretion activity has only been partly elucidated. Previous in vitro and human studies revealed chain length-dependent mechanisms, in which fatty acids having 12 carbon chain length or more trigger CCK secretion [5-8]. However, further structural features that determine the CCK-releasing activity of fatty acids have not been clarified.

Understanding the nutrient sensing mechanisms of enteroendocrine cells is fundamentally important in physiology, but the translational relevance of these mechanisms is also significant. If the oral administration of specific compounds has potent and selective stimulatory effects on target enteroendocrine cells, it will be possible to control physiological reactions, such as postprandial glycemia, lipidemia, and appetite by enhancing specific gut hormone secretion.

Recently, fatty acid derivatives (hydroxy- or oxo-fatty acids) produced by gut lactic acid bacteria (*Lactobacillus plantarum*) [9] have attracted a lot of attention for their favorable biological functions in both cell and animal models. Specific fatty acid derivatives can exert anti-inflammatory effects, cytoprotective effects, modify lipid/energy metabolism, and protect gut barrier function [9-15]. However, no current research is focused on the effect of those fatty acids on enteroendocrine function.

Fatty acid derivatives produced by gut lactic acid bacteria are a valuable tool to explore the relationship between the structure and activity of various fatty acids. In the present study, we aimed to reveal the structural features of fatty acids that directly and potently stimulate gut hormone secretion from enteroendocrine cells. CCK secretion was examined in response to more than 30 fatty acids in a murine CCK-producing enteroendocrine cell line, STC-1. We identified two specific structures responsible for potent CCK releasing activity in these fatty acids, and further investigated the cellular mechanism and in vivo effects in rats.

MATERIAL AND METHODS

Materials

Fatty acid metabolites produced by gut lactic acid bacteria [9] (Supporting Information Table S1) were provided by NITTO Pharmaceutical Industries, Ltd (Kyoto, Japan). All of fatty acids had more than 90% (mostly, more than 95%) purity (Table S1). Cell culture consumables (DMEM, fetal bovine serum, and penicillin/streptomycin) were purchased from Invitrogen (Carlsbad, CA, USA). Trypsin-EDTA solution, HEPES, and acetaminophen were purchased from Sigma (St. Louis, MO, USA). A GPR40 antagonist, GW1100, was purchased from Cayman Chemical (Ann Arbor, MI, USA), and a GPR120 antagonist,

AH7614, was purchased from Focus Biomolecules (Plymouth Meeting, PA, USA). Unless specified, all other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

CCK secretion study in STC-1 cells [16, 17]

STC-1 cells (a gift from Dr. Hanahan, University of California, San Francisco, CA) were grown in DMEM (Invitrogen, Cat. No. 12100–038) supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 500 μg/mL of streptomycin. The cells were incubated in a humidified, 5% CO_2 atmosphere at 37°C. Cells were routinely subcultured by trypsinization upon reaching 80–90% confluency. Cells at passage numbers 20–40 were used for experiments.

STC-1 cells were seeded in 48-well culture plates at a density of 1.25×10^5 cells/well and grown for 2–3 days until reaching 80–90% confluency. Cells were washed twice with HEPES buffer (140 mM NaCl, 4.5 mM KCl, 20 mM HEPES, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 0.1% BSA, pH 7.4) to remove the culture medium and then exposed to the test agents dissolved in HEPES buffer for 60 min at 37°C. Since the fatty acids were initially dissolved in ethanol, all test agents contained ethanol at a final concentration of 0.1% (v/v). Following incubation, the supernatants were collected and centrifuged at 800 × *g* for 5 min at 4°C to remove any cells. The supernatants were then stored at -50°C until the CCK concentration was measured using a commercially available enzyme immunoassay (EIA) kit (EK-069-04, Phoenix Pharmaceuticals Inc., Belmont, CA). The primary antiserum provided in this kit cross-reacts (100%) with sulfated and nonsulfated CCK (26–33), CCK-33 (porcine), caerulein, gastrin-1 (human), and big gastrin-1 (human). The antiserum also cross-reacts (12.6%) with CCK (30–33); however, there is 0% cross-reaction with

pancreatic polypeptide (human) and vasoactive intestinal peptide (including human, porcine, and rat). Because STC-1 cells do not express detectable levels of gastrin [5], we used the EIA kit to measure CCK. The coefficients of the intra- and interassay variation were <10% and <15%, respectively.

Measurement of cytotoxicity in STC-1 cells

The cytotoxic effects of the fatty acids on STC-1 cells were determined by measuring the release of lactate dehydrogenase (LDH) from the cell into the supernatant. STC-1 cells were exposed to test agents, as described above. LDH was measured using a cytotoxicity detection kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Cytotoxicity was calculated as the relative release (%) of LDH after exposure to the test agents compared to the total LDH (100%) released upon treatment with lysis reagent.

Animals experiments

Male Sprague–Dawley rats (7-weeks-old) were purchased from Japan SLC (Hamamatsu, Japan). The experiments were performed in a temperature-controlled room maintained at 23 ± 2°C with a 12 h light-dark cycle (8:00–20:00 light period). All animals had free access to water and were fed a semi-purified diet containing 25% casein, based on an AIN-93G diet [18], for 4–6 days as an acclimation period, and then divided into test groups based on body weight. Rats were fasted overnight the day before the experiment. The study was approved by the Hokkaido University Animal Ethics Committee, and the animals were maintained in accordance with the guidelines for care and use of laboratory animals at Hokkaido University (permission no. 14-0013).

154 Acetaminophen Test

155 Test agents were suspended in vehicle (saline containing 1.5% (w/v) carboxymethyl
156 cellulose (CMC) and 1% (w/v) acetaminophen). Acetaminophen (100 mg/kg body weight)
157 was added as an absorbable marker to assess the gastric emptying rate [19, 20]. A
158 diunsaturated aldehyde, *trans,trans*-2,4-decadienal (2t,4t-decadienal, Tokyo Chemical
159 Industry Co., Ltd., Tokyo, Japan) was employed as a positive control, as it has an inhibitory
160 effect on gastric emptying in rats [21].

161 Test suspensions containing fatty acids or vehicle were orally administered at a dose
162 of 10 mL/kg body weight through a feeding tube (5 Fr, Atom Medical Co., Tokyo, Japan).
163 Tail vein blood samples (60 µL) were collected prior to (0 min) and 15, 30, 45, 60, 90, and
164 120 min after oral administration. Blood samples were immediately mixed with heparin
165 (final concentration at 50 IU/mL, Nacalai Tesque, Inc., Kyoto, Japan) on ice. Plasma was
166 separated from the blood by centrifugation at 2300 × *g* for 10 min at 4°C, and then frozen at
167 -80°C until analysis. Plasma acetaminophen concentrations were measured using an
168 acetaminophen detection kit (Kanto Chemical Co., Inc., Tokyo, Japan).

169

170 Phenol Red Test

171 The effects of fatty acids on gastric emptying rate were further assessed using an
172 unabsorbable marker, phenol red [22, 23]. Phenol red (5 mg/kg body weight) was added to
173 both test suspensions and vehicle controls. Fifteen minutes after oral administration, rats
174 were euthanized by exsanguination under isoflurane anesthesia (MSD K.K., Tokyo, Japan).
175 The stomach was removed after cramping both proximal and distal sites of the tissue. The
176 stomach content was flushed twice with cold saline, and the washout solution was collected.
177 The debris was removed by centrifugation at 8400 × *g* for 10 min at 4°C. After adding 1 N

NaOH to the supernatant (1/10 volume of the supernatant), the concentration of phenol red was measured spectrophotometrically at 560 nm. The gastric emptying rate was calculated as follows:

Gastric emptying rate (%)

= [{the amount of phenol red administrated (mg) – the amount of phenol red remaining in the stomach (mg)}/the amount of phenol red administrated (mg)] x 100

Statistical analysis

Results are expressed as mean ± SEM. Significant differences among the control (vehicle) treatment and test groups were determined using Dunnett's post-hoc test ($P < 0.05$) as described in the figure legends.

RESULTS

The CCK releasing activity of 33 fatty acids was examined in STC-1 cells (Fig. 1A-D). Out of these, 7 had significant stimulatory effects on CCK release, especially 10-oxo-trans-11-octadecenoic acid (10-oxo-11t-18:1) (Fig. 1A and C), 13-oxo-cis-9,cis-15-octadecadienoic acid (13-oxo-9c,15c-18:2) (Fig. 1B and D), 10-oxo-cis-6,cis-12-octadecadienoic acid (10-oxo-6c,12c-18:2) (Fig. 1D), 10-oxo-cis-6,trans-11-octadecadienoic acid (10-oxo-6c,11t-18:2) (Fig. 1D), and 10-oxo-trans-11,cis-15-octadecadienoic acid (10-oxo-11t,15c-18:2) (Fig. 1D). Out of these 5 fatty acids, 13-oxo-9c,15c-18:2 and 10-oxo-6c,12c-18:2 had a common single oxo residue located between two *cis*-double bonds, while the remaining 3 fatty acids (10-oxo-11t-18:1, 10-oxo-6c,11t-18:2, 10-oxo-11t,15c-18:2) had a common

202 “α,β,-unsaturated carbonyl” or “enone” structure (Fig. 1E).

203 Both 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2 stimulated CCK secretion in a
204 dose-dependent manner (Fig. 2A and B). A significant increment in CCK secretion was
205 observed with ≥100 μM of 10-oxo-11t-18:1, and ≥50 μM of 13-oxo-9c,15c-18:2. Five fatty
206 acids with potent CCK-releasing activity (Fig. 1) did not exert cytotoxic effects in STC-1
207 cells, as evaluated by LDH release assay (Fig. 2C).

208 We examined the involvement of the major fatty acid receptors GPR40 [24, 25] and
209 GPR120 [26] as potential sensors of 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2.
210 Pretreatment with a GPR40 antagonist (GW1100) abolished CCK secretion induced by
211 both fatty acids (Fig. 3A and B). In contrast, treatment with increasing concentrations
212 (50-200 μM) of a GPR120 antagonist (AH7614) did not affect CCK secretory responses to
213 both fatty acids (Fig. 3C).

214 The effect of oral administration of the oxo-fatty acids on gastric emptying rate was
215 examined in vivo by oral co-administration of a test agent and an absorbable marker,
216 acetaminophen (Fig. 4A, C, 5B), or a non-absorbable marker, phenol red (Fig. 4B). An
217 unsaturated aldehyde 2t,4t-decadienal, used as a positive control [21], significantly
218 reduced the appearance of acetaminophen in the peripheral vein (Fig. 4A) and the gastric
219 emptying rate (Fig. 4B). At the same dose (25 mg/kg), 13-oxo-9c,15c-18:2 administration
220 resulted in significantly lower acetaminophen levels after 15 min (Fig 4A), and lower gastric
221 emptying rates (Fig. 4B), while 10-oxo-11t-18:1 administration had no effect compared to
222 the control (vehicle) treatment. A dose-response study (Fig. 4C) confirmed the suppressive
223 effect of oral 13-oxo-9c,15c-18:2 administration on gastric emptying rate, for doses of more
224 than 23.6 mg/kg (80 μmol/kg).

225 The effect of 13-oxo-9c,15c-18:2 on CCK secretion in STC-1 cells and on gastric

emptying rate in rats were further compared with those of other fatty acids sharing partially common structures (12-oxo-9c-18:1 and 13-hydroxy-9c,15c-18:2). As shown in Fig. 5A, 13-oxo-9c,15c-18:2 significantly increased CCK secretion in STC-1 cells, while other fatty acids had no effect. No stimulatory effects of 13-hydroxy-9c,15c-18:2 were reproduced (Fig. 1B). Plasma acetaminophen concentrations were significantly lower in rats administered 13-oxo-9c,15c-18:2 (160 μmol/kg = 47 mg/kg), until 60 min after oral administration, compared to that of rats administered the vehicle only. Oral administrations of the same dose (160 μmol/kg = 47 mg/kg) of 12-oxo-9c-18:1 or 13-hydroxy-9c,15c-18:2 did not affect the acetaminophen response observed in vehicle-treated rats.

DISCUSSION

Dietary fatty acids are known to be potent stimulants for CCK secretion. Chain length-dependent CCK secretion has been demonstrated previously both in in vivo and in vitro studies [5-8]. A previous study demonstrated differences in the CCK-releasing potency of various fatty acids [27], however, the structural features of fatty acids with potent CCK-releasing activity remain unclear. In the present study, using hydroxy- and oxo-fatty acids produced by gut lactic acid bacteria as metabolites, we identified two specific structures responsible for the potent CCK-releasing activity. The stimulatory effects of these fatty acids are mediated by a fatty acid receptor, GPR40. Further in vivo studies demonstrated that a single oral administration of a fatty acid possessing the specific structure exerted inhibitory effect on gastric emptying in rats. These findings revealed novel specific structure(s) in fatty acids that are responsible for stimulating CCK secretion in an enteroendocrine cell model, and for reducing gastric emptying rate.

In the present study, we tested more than 30 octadecanoic (C18) fatty acids for

CCK-releasing activity to elucidate any specific features involved in the activity. Interestingly, most of the fatty acids tested had almost no effect on stimulating CCK secretion. The degree of unsaturation, positions of double bonds, cis/trans orientation, conjugation, and positions of the hydroxy- or oxo-group are not apparently linked to CCK-releasing activity. Overall, the oxo-fatty acids had more potent activity than the hydroxy-fatty acids, and 5 oxo-fatty acids had an apparent stimulatory effect at the test concentration of 100 μM without cytotoxic effects (Fig. 2C). These results indicate that fatty acids possessing these specific structures (Fig. 1E) have potent CCK releasing activity in STC-1 cells. Apart from these potently stimulatory fatty acids, 10-oxo-6c-18:1 and 10-hydroxy,13-hydroxy-6c-18:1 had mild stimulatory effects (Fig. 1C), suggesting that there are other factors involved in their CCK-releasing activity. There are several limitations in STC-1 cells. The cell line has different properties from native mouse intestinal CCK cells [28], and co-produces other gut hormones such as GLP-1 and GIP [29]. Thus, the CCK-releasing activity of certain material found in STC-1 cells is not necessarily observed in animal/human study after oral administration. However, the cell line is still useful model for studying nutrient sensing mechanism in enteroendocrine cells.

The fatty acids tested in the present study are not generally recognized as dietary fatty acids because they are produced as metabolites by gut lactic acid bacteria [9]. Some of fatty acid metabolites were detected in the intestinal tissue and plasma of mice [9], and *Lactobacillus plantarum* could survive in the intestine, but the concentrations of fatty acid metabolites (10-5000 pg/100 mg or pg/100 μL range) were apparently lower than the doses used in the present study. The structures identified in the present study would therefore not be directly related to CCK secretion in response to dietary fat ingestion. However, our findings provide a novel insight into the chemical sensing mechanisms in enteroendocrine

274 cells. These structures could be utilized as key molecular features for stimulating gut
275 hormone secretion.
276 The stimulatory effect of 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2 were abolished by a
277 GPR40 antagonist (Fig. 3A and B), but not by a GPR120 antagonist (Fig. 3C), suggesting
278 that the CCK release is preferably mediated by GPR40 (Fig. 1E). GPR40 and GPR120 are
279 well known as receptors for medium-long chain fatty acids [30, 31]. Except carbon chain
280 length dependency, the structure-activity relationships of fatty acid properties such as
281 degree of unsaturation, position or cis-trans isomerism of double bond(s) have not been
282 established. Although various GPR40 agonists has been developed [32, 33], none of them
283 possess the structures found in the present study. The present study revealed two distinct
284 structures related to potent activation of GPR40-mediated CCK secretion in
285 enteroendocrine cells. A recent study has found that fatty acids with an α,β,-unsaturated
286 carbonyl (enone) structure have an anti-inflammatory effect in adipocytes [15]. The
287 possible involvement of peroxisome proliferator-activated receptors and GPCRs were
288 discussed, but the involvement of GPR40 was not demonstrated.
289 A previous paper [11] demonstrated that 10-hydroxy-12c-18:1 protected the tight
290 junction barrier via GPR40 in Caco2 cells. In the present study, we found that CCK
291 secretion was not affected by the fatty acid (Fig. 1A), suggesting that the fatty acid requires
292 other factors (time, intracellular signal transduction pathway, etc.) in a specific cellular
293 response for the observed activity through GPR40.
294 We expected that the fatty acids would exert an inhibitory effect on gastric emptying in
295 vivo, as delaying gastric emptying is a distinct physiological function of several gut
296 hormones including CCK [3, 4, 34]. Although the effect was relatively smaller than that of
297 2t,4t-dacadienal [21] at the same weight dose (25 mg/kg), the oral administration of

298 13-oxo-9c,15c-18:2 decreased the gastric emptying rate as evaluated by two independent
299 methods, while 10-oxo-11t-18:1 did not (Fig. 4A and B). Because the fatty acids were orally
300 administered, the failure of 10-oxo-11t-18:1 to inhibit gastric emptying could be due to
301 insufficient delivery of active form fatty acids to the site of action (possibly the small
302 intestinal lumen). It is possible that the fatty acid was rapidly degraded or absorbed in the
303 stomach or intestinal lumen, or adhered to the stomach mucosa. However, based on our
304 results it appears that 13-oxo-9c,15c-18:2 successfully reach the small intestinal lumen
305 following oral administration, resulting in a reduced gastric emptying rate possibly through
306 stimulation of CCK-producing enteroendocrine cells. Further studies are needed in the
307 future to investigate which factors (CCK and/or other gut hormones, such as GLP-1, PYY,
308 serotonin, etc.) are involved in the effect of 13-oxo-9c,15c-18:2 in vivo. In addition, the
309 nutritional properties of these modified fatty acids, including their digestion, absorption, and
310 metabolism, have not yet been characterized. Such information would help to explain the
311 differences in the observed results between in vitro and in vivo experiments.
312 In the case of 13-oxo-9c,15c-18:2, the oxo-group is essential for the CCK releasing
313 and gastric emptying inhibitory effect (Fig. 5), since 13-hydroxy-9c,15c-18:2 had no effect.
314 This result suggests that fatty acids without in vitro activity consistently have no effect on
315 gastric emptying rate in vivo. It is interesting that the in vitro CCK-releasing activity
316 correlates well with the observed in vivo gastric inhibiting effect.
317 Recent studies have revealed the physiological functions of fatty acid metabolites
318 produced by gut lactic acid bacteria [9-15]. Some of these metabolites were found in the
319 mouse colon [9], as gut microbiota is located primarily in the distal intestine. Continuous
320 ingestion of substrate fatty acids for gut lactic acid bacteria may provide beneficial effects
321 through increased gut hormone secretion induced by specific luminal fatty acids, such as

13-oxo-9c,15c-18:2 and 10-oxo-11t-18:1. However, the efficacy of digestion or amount of metabolic products produced in the gut is not yet predicable. Therefore, oral administration of fatty acids synthesized in vitro rather than a dietary co-supplementation of lactic acid bacteria and substrate fatty acids could be applicable for reducing glycemia through suppression of gastric emptying [35], and for reducing appetite through increased secretion of gut hormones including CCK, peptide-YY, and glucagon-like peptide-1.

In conclusion, by examining various fatty acids produced by gut lactic acid bacteria, we have identified two specific structures responsible for potent CCK secretion in enteroendocrine cells. The stimulation of CCK secretion was probably mediated by a fatty acid receptor, GPR40. A fatty acid with the specific structure exerted an inhibitory effect on gastric emptying after an oral administration in rats. These results revealed a novel fatty acid-sensing mechanism in enteroendocrine cells.

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Author contributions

SO, AY, MK, XL and TH performed the experiments;

TH, SN, MS, TY, and HH designed and conceived the experiments;

SN, MS, TY, SK and JO provided the materials.

Conflict of interest

Saki Nishimura, Masayoshi Sakaino, and Takatoshi Yamashita are employee of J-Oil Mills, Inc.

Figure legends

Fig. 1.

CCK releasing activities of various fatty acids derived from lactic acid bacteria (A-D), and common structures identified in oxo-fatty acids with potent CCK-releasing activity (E). STC-1 cells were exposed to fatty acids (100 μM) for 60 min at 37°C. The concentration of CCK in the supernatant was measured using a commercial CCK-ELISA kit. Values are expressed as means and SEM (n=3-4). Plus (+) signs indicate significant differences compared to the vehicle treatment ($P < 0.05$ by Dunnett's test).

Fig. 2.

Dose-response effects of 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2 on CCK secretion (A, B), and cytotoxic effects of fatty acids with potent CCK-releasing activity (C).

STC-1 cells were exposed to various concentrations of 10-oxo-11t-18:1 or 13-oxo-9c,15c-18:2 for 60 min at 37°C, and CCK concentrations in the supernatant were measured (A, B). Values are expressed as means and SEM (n=3-4). Plus (+) signs indicate significant differences compared to the vehicle treatment ($P < 0.05$ by Dunnett's test). LDH activity in the supernatant was measure after 60 min exposure to fatty acids (100 μM). For the total LDH activity control, a lysis reagent was used to release all intracellular LDH. The values represent LDH activity (%) relative to the total LDH control and are expressed as means with SEM of cells in three wells.

Fig. 3.

Effects of GPR40 or GPR120 antagonists on CCK secretion induced by 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2.

STC-1 cells were pre-treated with a GPR40 antagonist (GW1100) (A and B) or a GPR120 antagonist (AH7614) (C) or its vehicle (0.1% DMSO) for 30 min, then exposed to 10-oxo-11t-18:1 (100 μM) or 13-oxo-9c,15c-18:2 (100 μM) for 60 min. Values are expressed as means and SEM (n=3-4). Plus (+) signs indicate a significant difference compared to the vehicle without antagonist ($P < 0.05$ by Dunnett's test). 'NS' represents no significant differences within 10-oxo-11t-18:1- or 13-oxo-9c,15c-18:2-treated cells, respectively.

Fig. 4.

The effects of oral administration of 10-oxo-11t-18:1 and 13-oxo-9c,15c-18:2 on gastric emptying rate in rats.

After overnight fasting, 25 mg/kg of 10-oxo-11t-18:1 (84.9 μmol/kg), 13-oxo-9c,15c-18:2 (84.3 μmol/kg), or 2t,4t-decadienal (164.2 μmol/kg) was orally administered with acetaminophen (A) or with phenol red (B). Blood samples were collected from the tail vein up to 120 min later, and plasma acetaminophen concentrations were measured (A). Different doses (40, 80 and 160 μmol/kg) of 13-oxo-9c,15c-18:2 were examined using the same experimental method (C). In a separate experiment, gastric contents were collected 15 min after oral co-administration of test agents and phenol red (B). The gastric emptying rate was calculated based on the amount of phenol red remaining in the stomach lumen. Values are expressed as means and SEM (n=4-6). Asterisks (*) indicate a significant difference compared to the vehicle treatment (B) at each time point (A, C) ($P < 0.05$ by

Dunnett's test).

Fig. 5.

CCK secretion in STC-1 cells and gastric emptying rate in rats in response to oral administration of 13-oxo-9c,15c-18:2 and structurally related fatty acids.

STC-1 cells were exposed 100 μM of fatty acids (13-oxo-9c,15c-18:2, 12-oxo-9c-18:1 or 13-hydroxy-9c,15c-18:2) for 60 min, and the concentrations in the supernatant were measured (A). Values are expressed as means and SEM (n=3-4). Plus (+) signs indicate significant differences in concentration compared to the vehicle treatment ($P < 0.05$ by Dunnett's test). Fatty acids (160 μmol/kg) or vehicle were orally administered to rats, and blood samples were collected from the tail vein. Acetaminophen concentrations were measured in the plasma. Values are expressed as mean and SEM (n=5-6). Asterisks (*) indicate significant differences in concentration compared to the vehicle treatment at each time point ($P < 0.05$ by Dunnett's test).

Figure 1

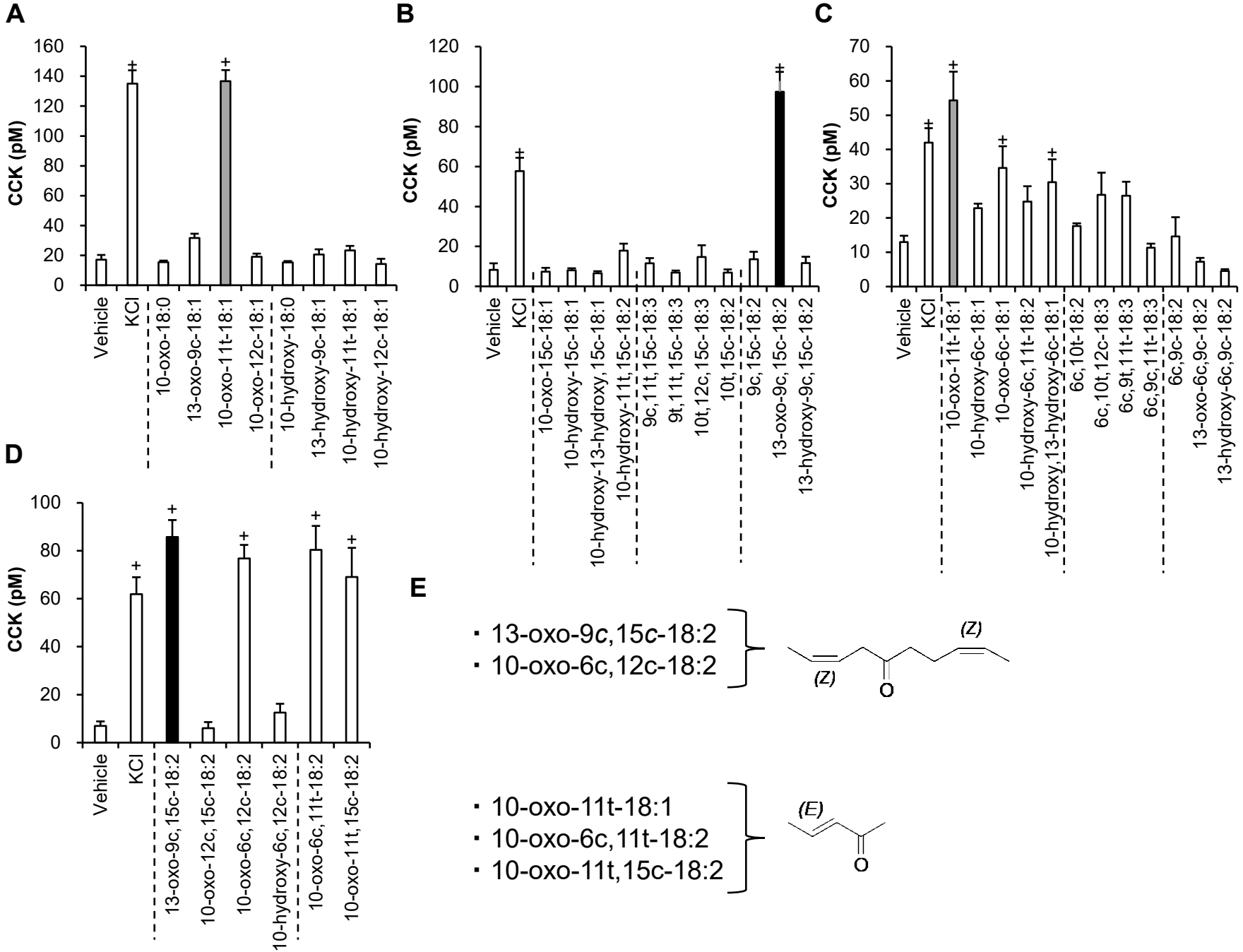

A
CCK (pM)
160
140
120
100
80
60
40
20
0
Vehicle
KCl
10-oxo-18:0
13-oxo-9c-18:1
10-oxo-11t-18:1
10-oxo-12c-18:1
10-hydroxy-18:0
13-hydroxy-9c-18:1
10-hydroxy-11t-18:1
10-hydroxy-12c-18:1
B
CCK (pM)
120
100
80
60
40
20
0
Vehicle
KCl
10-oxo-15c-18:1
10-hydroxy-15c-18:1
10-hydroxy-13-hydroxy,15c-18:1
10-hydroxy-11t,15c-18:2
9c,11t,15c-18:3
9t,11t,15c-18:3
10t,12c,15c-18:3
10t,15c-18:2
9c,15c-18:2
13-oxo-9c,15c-18:2
13-hydroxy-9c,15c-18:2
C
CCK (pM)
70
60
50
40
30
20
10
0
Vehicle
KCl
10-oxo-11t-18:1
10-hydroxy-6c-18:1
10-oxo-6c-18:1
10-hydroxy-6c,11t-18:2
10-hydroxy,13-hydroxy-6c-18:1
6c,10t-18:2
6c,10t,12c-18:3
6c,9t,11t-18:3
6c,9c,11t-18:3
6c,9c-18:2
13-oxo-6c,9c-18:2
13-hydroxy-6c,9c-18:2
D
CCK (pM)
100
80
60
40
20
0
Vehicle
KCl
13-oxo-9c,15c-18:2
10-oxo-12c,15c-18:2
10-oxo-6c,12c-18:2
10-hydroxy-6c,12c-18:2
10-oxo-6c,11t-18:2
10-oxo-11t,15c-18:2
E
・13-oxo-9c,15c-18:2
・10-oxo-6c,12c-18:2
(Z)
(Z)
O
・10-oxo-11t-18:1
・10-oxo-6c,11t-18:2
・10-oxo-11t,15c-18:2
(E)
O

Figure 2

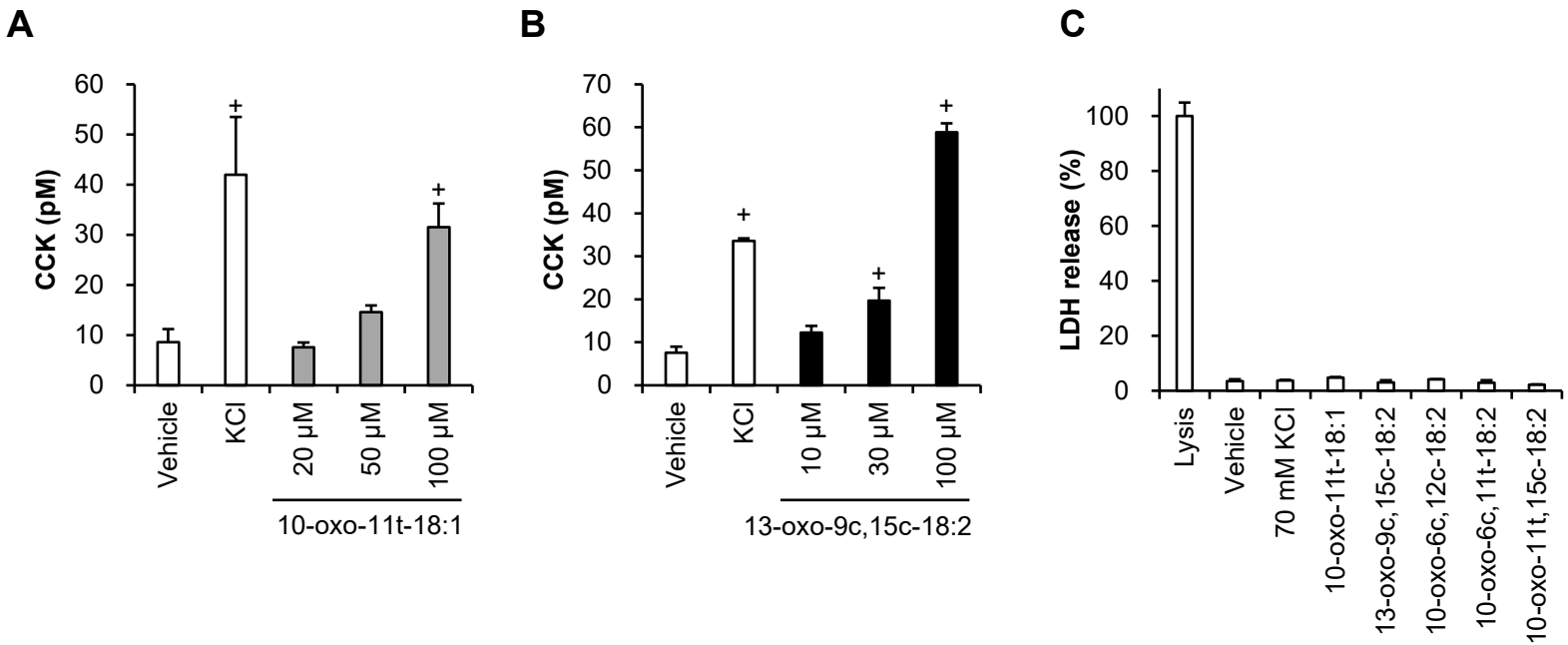

A
CCK (pM)
60
50
40
30
20
10
0
+
+
Vehicle
KCl
20 µM
50 µM
100 µM
10-oxo-11t-18:1
B
CCK (pM)
70
60
50
40
30
20
10
0
+
+
+
Vehicle
KCl
10 µM
30 µM
100 µM
13-oxo-9c,15c-18:2
C
LDH release (%)
100
80
60
40
20
0
Lysis
Vehicle
70 mM KCl
10-oxo-11t-18:1
13-oxo-9c,15c-18:2
10-oxo-6c,12c-18:2
10-oxo-6c,11t-18:2
10-oxo-11t,15c-18:2

Figure 3

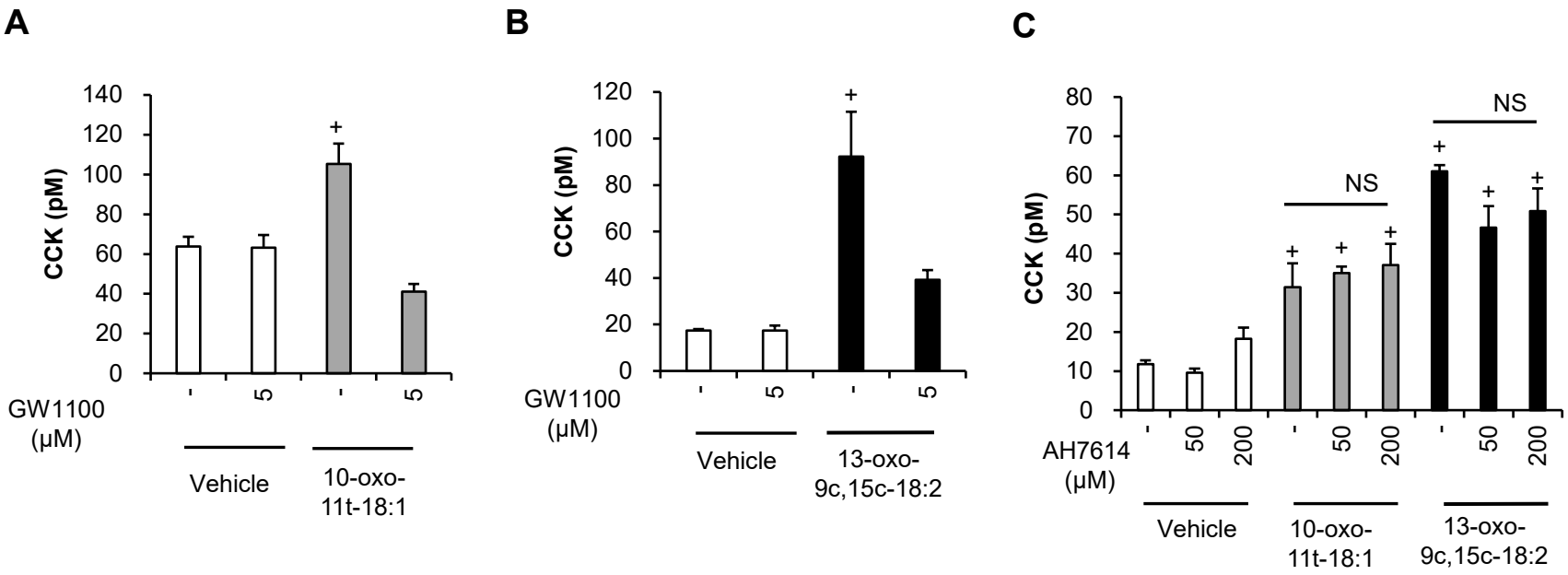

A
CCK (pM)
140
120
100
80
60
40
20
0
+
GW1100 (μM)
-
5
-
5
Vehicle
10-oxo-11t-18:1
B
CCK (pM)
120
100
80
60
40
20
0
+
GW1100 (μM)
-
5
-
5
Vehicle
13-oxo-9c,15c-18:2
C
CCK (pM)
80
70
60
50
40
30
20
10
0
NS
NS
+
AH7614 (μM)
-
50
200
-
50
200
-
50
200
Vehicle
10-oxo-11t-18:1
13-oxo-9c,15c-18:2

Figure 4

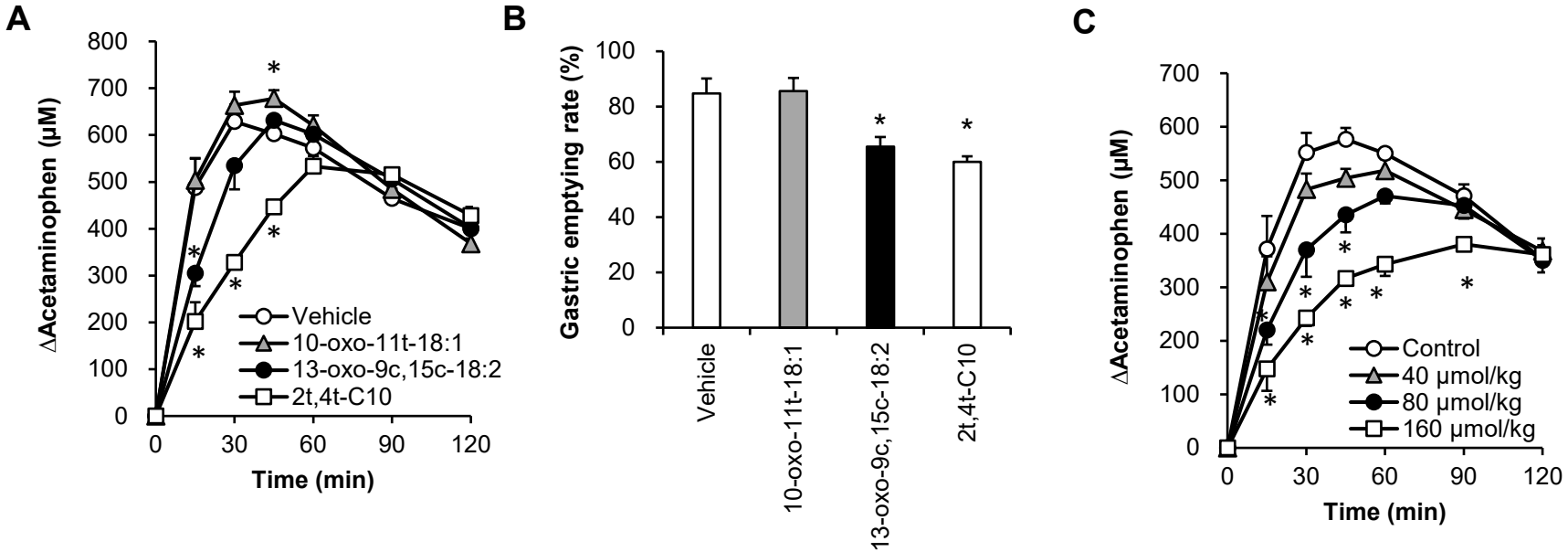
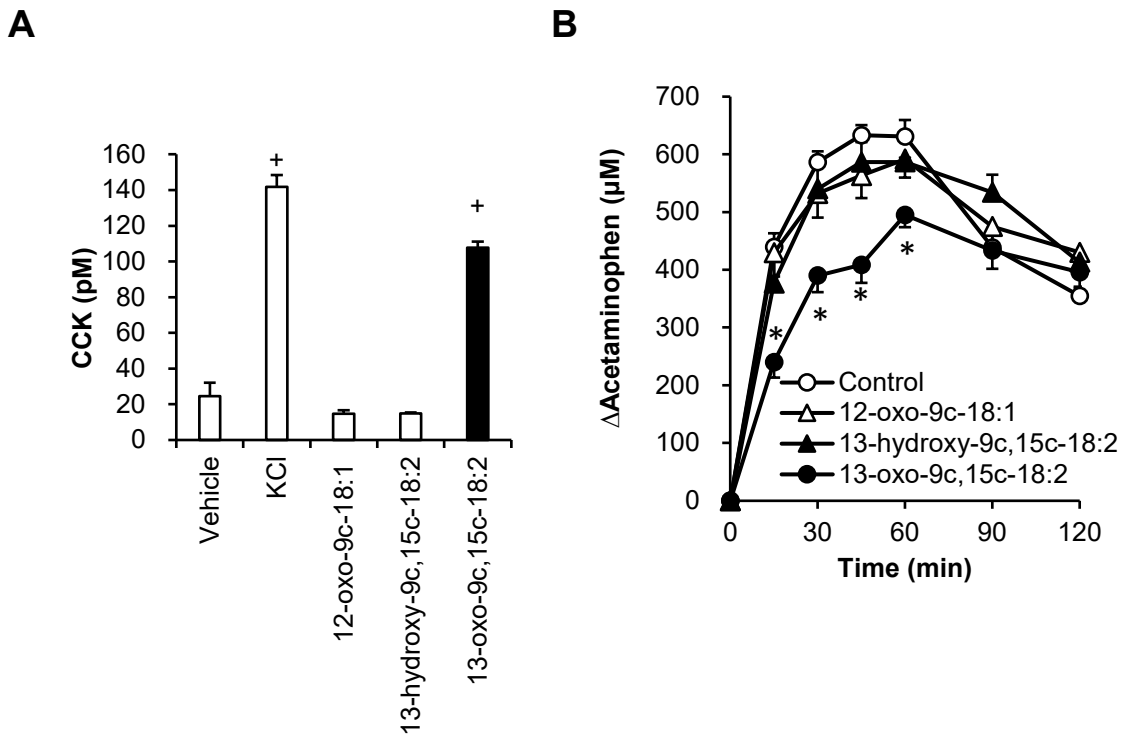
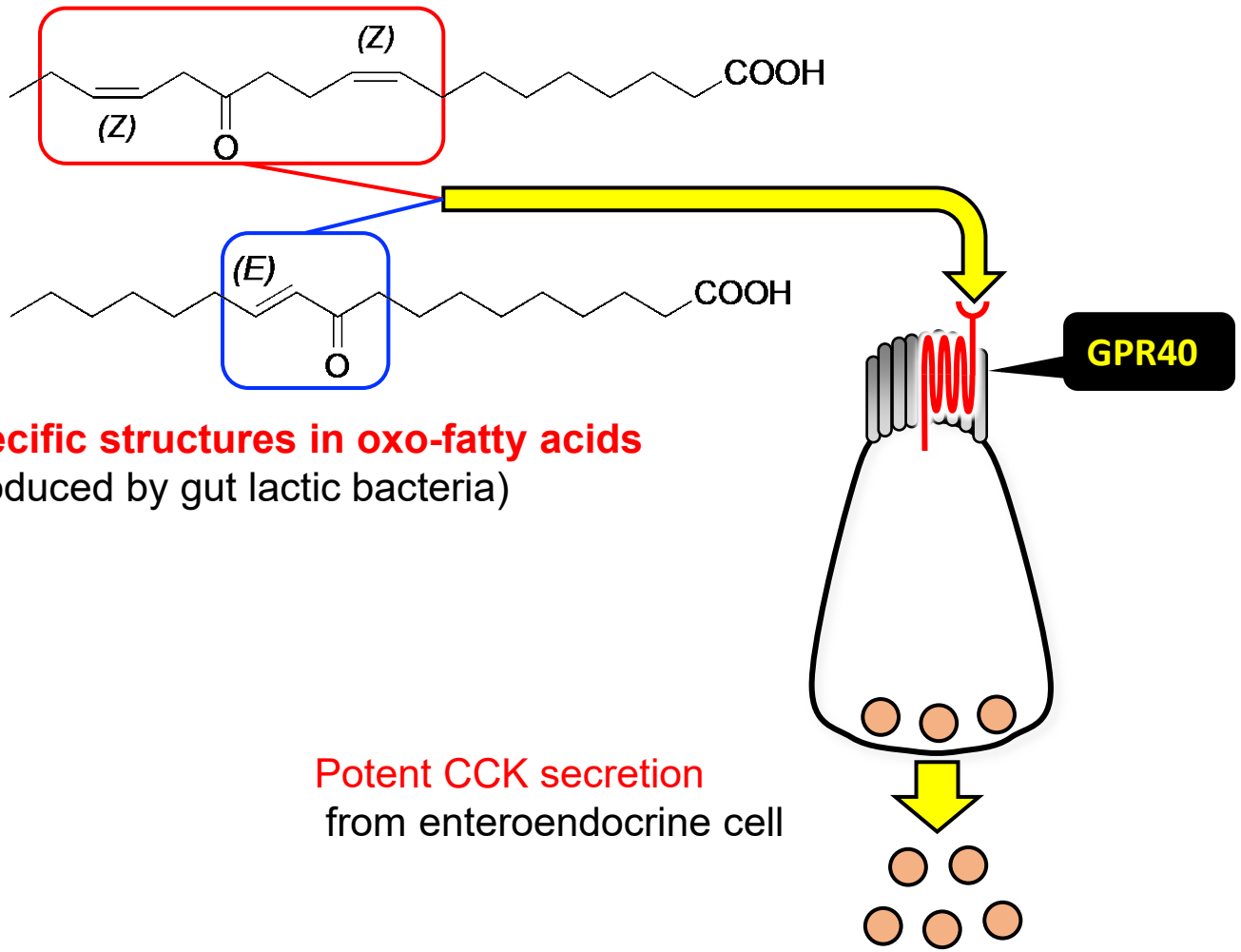

A
ΔAcetaminophen (μM)
800
700
600
500
400
300
200
100
0
0
30
60
90
120
Time (min)
Vehicle
10-oxo-11t-18:1
13-oxo-9c,15c-18:2
2t,4t-C10
B
Gastric emptying rate (%)
100
80
60
40
20
0
Vehicle
10-oxo-11t-18:1
13-oxo-9c,15c-18:2
2t,4t-C10
C
ΔAcetaminophen (μM)
700
600
500
400
300
200
100
0
0
30
60
90
120
Time (min)
Control
40 μmol/kg
80 μmol/kg
160 μmol/kg

Figure 5


A
CCK (pM)
160
140
120
100
80
60
40
20
0
+
+
Vehicle
KCl
12-oxo-9c-18:1
13-hydroxy-9c,15c-18:2
13-oxo-9c,15c-18:2
B
ΔAcetaminophen (μM)
700
600
500
400
300
200
100
0
0
30
60
90
120
Time (min)
Control
12-oxo-9c-18:1
13-hydroxy-9c,15c-18:2
13-oxo-9c,15c-18:2
*
*
*
*

Graphic abstract