Physiology & Behavior 234 (2021) 113315

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Post-oral fat-induced satiation is mediated by endogenous CCK and GLP-1 in a fat self-administration mouse model

Vasiliki Vana [a,c,*], Michelle K. Lærke [a], Karen Kleberg [a], Piotr A. Mroz [b], Birgit L. Lindberg [a], Jeppe H. Ekberg [c], Jens F. Rehfeld [d], Thue W. Schwartz [c], Harald S. Hansen [a]

[a] Department of Drug Design and Pharmacology, University of Copenhagen, Jagtvej 160, DK-2100 Copenhagen, Denmark
[b] Department of Chemistry and Gill Center for Biomolecular Science, Indiana University, Bloomington, 800 E. Kirkwood Ave. A563, United States
[c] Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, DK-2200 Copenhagen, Denmark
[d] Department of Clinical Biochemistry (KB3011), Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

ARTICLE INFO

Keywords:
Fat
GLP-1 receptor
CCK1 receptor
Satiation
Y_2 receptor
Jant4-C16

ABSTRACT

Triacylglycerol is the most abundant dietary lipid, and a strong stimulator of satiation. Absorption of triacylglycerol in the small intestine occurs in the form of free fatty acids and 2-monoacylglycerol, a process known to trigger not only the release of cholecystokinin (CCK) but also glucagon-like peptide 1 (GLP-1) and peptide YY (PYY). It remains controversial, however, whether endogenously released GLP-1 and PYY are required for fat-induced satiation. Using a self-administration model where mice are trained to self-administer Intralipid 30% intragastrically, we show that blocking the CCK_1 receptors with intraperitoneal devazepide diminishes the post-oral satiation effect of ingested fat. Similarly, s.c. administration of a GLP-1 receptor antagonist with a prolonged half-life (Jant4-C16) also reduced the post-oral satiation effect of ingested fat. Importantly, coadministration of the GLP-1 antagonist together with devazepide increased fat self-infusions to a level equal to the combined blockade of each individual peptide action alone, indicating an additive effect of endogenous CCK and GLP-1 in fat satiation signaling. Blocking the PYY Y_2 receptor did not further enhance the fat intake in devazepide-treated mice. Consistent with the above, we show that voluntary post-oral ingestion of fat increases CCK and GLP-1 plasma levels and is correlated positively with CCK and GLP-1 plasma concentrations. Taken together, our results support the role of endogenous GLP-1 in the regulation of fat intake and suggest that both CCK and GLP-1 are required for the fat satiation signaling.

1. Introduction

Obesity is an increasing problem worldwide [1]. The fundamental cause of obesity is an energy imbalance between calories consumed and expended. The consumption of energy-dense foods, which are typically high in fat content, is a significant contributor to the increasing prevalence of overweight and obesity [2] while fat has been recently identified as the principle regulator of energy intake among solid form macronutrients and across different mouse strains [3,4]. Thus, a better understanding of the physiological mechanisms controlling fat intake is important in order to get a better picture of the causes of obesity.

Satiation is the feeling of fullness that leads to termination of food intake. It has been shown that the gastrointestinal tract plays an important role in regulating acute and long-term energy intake in the absence of oral cues. Food ingestion triggers gastrointestinal signals that regulate food intake and coordinate nutrient digestion and absorption [5]. The stomach sends satiation signals that are primarily activated by mechanical distension, whereas the signals from the intestine derive from both mechanical and nutrient sensing [6–8]. Intestinal endocrine cells sense nutrients and release several peptides, many of which can delay gastric emptying and induce satiation. These peptide signals can also modulate vagal inputs or act directly via the blood on the brain appetite center [9]. While GLP-1 has a well-established effect on insulin release and even though the number of approved GLP-1 analogues for human use is increasing, the physiological role of endogenous GLP-1 in the regulation of food intake is unclear [10–12]. A GLP-1 analogue that can cross the blood brain barrier has been shown to decrease food intake and body weight in humans [13]. However, the endogenous GLP-1 is

* Corresponding author.
E-mail addresses: Vasiliki.vana@sund.ku.dk, hsh@sund.ku.dk (V. Vana), hsh@sund.ku.dk (M.K. Lærke), hsh@sund.ku.dk (K. Kleberg), hsh@sund.ku.dk (B.L. Lindberg), hsh@sund.ku.dk (H.S. Hansen).

https://doi.org/10.1016/j.physbeh.2021.113315
Received 4 October 2020; Received in revised form 6 January 2021; Accepted 7 January 2021
Available online 15 January 2021

rapidly degraded; thus, it is believed that intestinal GLP-1 must act via the vagus nerve [14]. Studies in rodents show controversial results of the role of endogenous GLP-1 in satiation [15–20] while administration of the GLP-1 receptor antagonist, exendin-(9–39), in humans caused an increase in GLP-1 blood levels without any effect on food intake [21,22].

We are studying the role of dietary fat in meal termination and the signaling from the gut to the brain [23]. Dietary fat can acutely induce dopamine release in the dorsal striatum [24–26], and this effect seems to be mediated by the fatty acid- moiety of dietary triacylglycerol, while the satiation effect can be mediated by both the fatty acid part as well as the 2-monoacylglycerol (2-MAG) part of digested triacylglycerol [27]. Depending upon the test paradigm used, the post-ingestive actions of fat can stimulate or suppress intake [24,28,29]. Pharmacological stimulation of GLP-1 receptors in the periphery seems to inhibit homeostatic chow intake, while hedonic fat intake seems to be inhibited by combined stimulation of GLP-1 receptors in both CNS and the periphery [30]. Fatty acids are known agonists of the free fatty acid 1 (FFA1) and free fatty acid 4 (FFA4) receptors, which both have been reported to be involved in GLP-1 release [5]. 2-MAG (including 2- oleoyl glycerol (2-OG)) is a GPR119 agonist that can release GLP-1 from enteroendocrine cells in humans and rodents [31–33]. In humans given a low dose of fat (20 mL olive oil), 2-OG was mainly responsible for the release of GLP-1, PYY, and neurotensin whereas the fatty acid part was mainly responsible for CCK release, while at higher doses of fat, fatty acids and 2-OG may act synergistically to induce hormone release from the small intestine [34,35].

In this study, we evaluated whether endogenous CCK, GLP-1, and PYY regulate post-oral self-induced fat satiation. Second, we aimed to determine the plasma levels of GLP-1 and CCK after voluntary post-oral intake in mice. We focused on a mouse model, where mice are trained to self-administer a lipid-emulsion directly into the stomach during a one-hour session each day [24,27]. The mice cannot see, smell, or taste the fat emulsions, but they can sense the concentration of fat entering the gastrointestinal system. Thus, we can investigate the post-oral effects of fat intake unbiased from flavor preferences, and measure the time course of intake. Moreover, with this model, we can measure directly the satiation effect of fat, rather than the satiety effect of a preload of fat, which is used in many studies.

2. Materials and methods

2.1. Ethical approval

The experiments were conducted at the University of Copenhagen and were approved by the Danish Committee for Animal Research (License 2013–15–2934–00766 C1). All experimental procedures were fully compliant with national and international accepted principles for the care and use of laboratory animals. 40 C57Bl6/J mice (male, 10–24 weeks of age) were used in the experiments. Mice were obtained from Janvier Labs (Le Genest-Saint-Isle, France). The mice were allowed to habituate for one week to the new environment. They were housed in a temperature (20 - 22 °C) and humidity (50 - 65%) controlled facility with a 12 / 12 h light-dark cycle before undergoing any surgical or behavioral procedures and had ad libitum access to standard chow (3.2 kcal g^{-1}, 14.5% energy fat, Altromin Spezialfutter GmbH & Co, Germany) and water unless otherwise described in the protocols below.

2.2. Gastric catheter implantation

The surgery was performed, as described previously [24]. Briefly, mice were anaesthetized with an intraperitoneal injection of ketamine/xylazine (100/15 mg kg^{-1}) or isoflurane, and the area between the shoulders and abdomen was shaved. Eye ointment was applied to both eyes to keep them hydrated, and analgesia was administered (5 mg kg^{-1} carprofen, s.c.). Following skin disinfection, two incisions were made, a small between the shoulders and one in the abdomen, app. 1.5 cm long. Catheter tubing (10 cm of Micro-Renathane tubing, MRE-033, 0.33 × 0.014 inches, Braintree Scientific, Braintree, MA, USA) was tunneled through the incision between the shoulders under the skin and into the abdomen via a hole through the abdominal muscle. The stomach was exteriorized, and a purse-string suture (7/0 Optilene, B. Braun Surgical S.A., Spain) allowed to tightly attach the tip of the catheter to the stomach wall and inside the lumen. Finally, the abdominal muscle and skin incisions were sutured.

2.3. Post-operative care

Following surgery, the mice were single-housed and had ad libitum access to standard chow and water. The first three days after surgery, they were treated with Rimadyl (carprofen 5 mg kg^{-1}) and a five-day recovery was allowed. The mice were monitored daily for any signs of stress or morbidity after surgery and until euthanisation. Mice showing signs of not being well were euthanized immediately.

2.4. Gastric glucose tolerance test (GGTT)

Mice with implanted gastric catheters received an intragastric bolus dose of glucose through the catheter (20% w/v, 10 mL kg^{-1}) following a six-hour fasting period. Prior to glucose, they were dosed s.c. with a long-acting GLP-1 receptor antagonist Jant-4 (9–40)a Lys40-C16 (Jant4-C16) [36] 23 h and 5 h in advance. Blood was sampled 20 min prior to the glucose bolus and at 0, 15, 30, 60, 90, and 120 min from the tail. Blood glucose concentration was immediately analyzed with a handheld glucose meter (Contour XT, Bayer Healthcare AG, Germany). The test was performed in a cross-over design with a week of wash-out in between tests.

2.5. Emulsion preparation

The mice were trained to self-administer Intralipid 30% (w/v) (IL30) (Baxter, Deerfield, IL, USA or Fresenius Kabi A/S, Copenhagen, Denmark) containing 1.2% (w/v) phospholipid and 1.7% (w/v) glycerol as emulsifiers. The calorie content of IL30 is 3 kcal mL^{-1}. To prepare the Intralipid 5% (IL5) emulsion, lecithin (Lipoid E PC S, Lipoid AG, Steinhausen, Switzerland) and glycerol (>99%, Sigma-Aldrich) were weighed off and thoroughly mixed before the Intralipid 30% was added in a 1:6 final dilution ratio. Thus, the final IL5 contained the same% (w/v) of phospholipids and glycerol as the IL30 emulsion but only 5% of triacylglycerol, which resulted in IL5 having a calorie content of 0.8 kcal mL^{-1}.

2.6. Behavioral apparatus

The mice were trained in operant test chambers enclosed in a sound-attenuating cubicle (MedAssociates, St. Albans, VT, USA). Each chamber was equipped with one sipper placed on the wall of the cubicle. All sippers were connected to a contact-based relay for lick detection ('contact lickometer'), that was programmed to trigger the activation of a connected infusion pump via TTL pulses.

2.7. Behavioral training protocol

Mice were trained daily in the operant chambers and learned to associate licking on a dry spout with intragastric infusions of IL30. They were presented with one spout always placed in the same position. To increase the mice's motivation to lick, they were calorie-restricted to 75% of normal chow intake for the duration of the behavioral experiments, receiving 2.6 g of chow each day after the 1 h session in the box. The food restriction also ensured a stable day-to-day intake during the sessions. Each dry lick was programmed to trigger an infusion that lasted for 6 s at a rate of 0.38 mL min^{-1}, which resulted in a 37.5 μL infusion. Licks detected while an infusion was taking place did not activate

additional infusions. A small amount of crushed chow was placed inside the sipper during the first 3–4 days of the training in order to increase the mice's interest in the sipper, and this allowed the mice to smell but not taste the chow. The training was continued until a stable baseline of infusions was achieved between five consecutive days, with less than 20% variation across days. The IL30 was then replaced with IL5, an increased number of infusions (minimum twofold increase) of IL5 was a confirmation of a trained mouse. Trained mice were sometimes used in more than one behavioral protocol given that a stable baseline was achieved prior to the experiment. The daily fat intake by the mice (coming from the chow plus the daily Intralipid intake) was calculated to represent 27.6% energy being a little lower than the 28.5 – 46.2% energy fat intake in the European population Table 1 [37].

2.8. Behavioral responses to CCK_1, Y_2, and GLP-1 receptor antagonists

When a stable baseline (five consecutive days of less than 20% variation in the number of IL30 infusions) was achieved, the effect of pharmacological agents was tested on the self-administration behavior. Jant4-C16 (GLP-1 receptor antagonist, 1 μmol kg^{-1}, 23 h and 5 h in advance, s.c.), devazepide (CCK_1 receptor antagonist, 2 mg kg^{-1}, 30 min in advance, i.p.), and the combination of the two were investigated in this set-up in a cross-over design ($n = 6$). The combination of devazepide (2 mg kg^{-1}, 30 min in advance, i.p.) and BIIE 0246 (Y_2 receptor antagonist, 2 mg kg^{-1}, 30 min in advance, i.p.) was tested in a cross-over design as well ($n = 7$). BIIE 0246 was dosed at a dose previously shown to inhibit the satiation effect of exogenously administered native PYY3–36 [38,39]. A minimum of five-days wash-out period was allowed between treatments and mice were habituated to vehicle injections for three days prior to testing.

2.9. Behavioral hormonal responses

Blood samples were collected from mice (n = 5–6) trained to self-administer IL30 (as described above). Blood was collected at the time point of the initiation of the scheduled one-hour session (the mice were euthanized immediately after blood collection) and after the one-hour session with IL30. Naïve, age-matched mice (n = 8) were included in this study. The mice were anaesthetized via isoflurane inhalation, and heart blood was collected into EDTA-coated vials and supplemented with 10 μl Dipeptidyl peptidase 4 (DPP4) inhibitor (Merck-Millipore, Billerica, MA, USA) and 500 kIU aprotinin (Sigma-Aldrich). Plasma was obtained by centrifugation at 10 000 g for 10 min at 4 °C within 20 min and immediately frozen on dry ice after separation. The samples were stored at −80 °C until further analysis.

One mouse was excluded during data analysis, as it gradually decreased the number of IL30 self-infusions over sessions and had a significantly lower number of infusions compared to the rest of the group (9 infusions versus 19.8 ± 3.9 infusions (n = 19).

2.10. Hormone analyses

All hormone analyses (n = 5–8) were performed on snap-frozen plasma and according to the manufacturer's protocol. Glucagon-like peptide 1 (GLP-1) total (GLP-17–36/37 and GLP-19–36/37) were measured using an ELISA kit (Total GLP-1 ver.2) from Meso Scale Discovery (Rockville, MD, USA). Electrochemiluminescence was measured using a SECTOR Imager 2400 instrument (Meso Scale Discovery), and data were analyzed using Discovery Workbench (Meso Scale Discovery). Cholecystokinin (CCK) was measured using a radioimmunoassay that reacts fully with mouse CCK peptides without cross-reactivity with any gastrins [40]. Plasma was mixed with barbital buffer added albumin (BBA), tracer (10 μCi ^{125}I-CCK8/20 mL BBA and antibody Ab92128), and incubated at 4 °C for 3–7 days. Separation of bound and un-bound CCK was performed by adding barbital buffer, 20% human plasma and 6 g active charcoal/100 mL (Sigma) for 15 min and subsequent centrifugation. Bound ^{125}I-CCK8 was counted in a Wallac Wizard 1470 gamma counter (GMI).

Table 1
Diet composition of the fat self-administration mouse model as compared to ad libitum chow diet.

Diet energy composition	Chow	Self-administration*
Total calorie intake in Kcal per day:	10.8	9.7
Total fat intake E%:	14.6	27.6
Total carbohydrate intake E%:	51.1	43.4
Total fiber intake E%:	5.8	4.9
Total protein intake E%:	28.5	24.2

* The diet composition of the self-administration model is calculated based on the regular chow intake and the one-hour self-administration of IL30.

2.11. Synthesis of the GLP-1 antagonist (Jant4–16)

The peptide was prepared as the C-terminus amide by automated Fmoc/*t*-Bu solid-phase methodology on Rink Amide resin (AAPPtec, Louisville, KY) employing 6-Cl-HOBt/DIC coupling chemistry on CSBio model CS336X peptide synthesiser. The protecting group scheme consisted of Arg(Pbf); Boc-N-Asp(O*t*Bu); Gln(Trt); Glu(O*t*Bt); Lys(Boc) or Lys(Mtt) for residue 40; Ser(*t*-Bu); Tyr(*t*-Bu) and Trp(Boc). Upon completion of main sequence synthesis, side-chain Mtt protection at residue Lys 40 was removed by treatment with 2% TFA, 2%TIS in DCM. Lipidation was performed on the resin with palmitic acid activated with PyBOP/DIPEA in DCM/NMP (1:3). The reaction was monitored by free amine detecting Kaiser test. All conventional residues were purchased from Midwest Biotech (Fisher, IN), 6-Cl-HOBt, PyBOP and DIC from AAPPtec (Louisville, KY), DIPEA from Sigma-Aldrich (St. Louis, MO). The peptide was cleaved from the resin and deprotected by treatment with TFA containing 2.5% TIS, 2.5% H_2O and 2.5% phenol then purified on a GLP-1 antagonist-dedicated C18 preparative PR-HPLC column (Vydac 218TP, 22 × 250 mm, 10μm) with 0.05% TFA/H_2O and 0.05% TFA/CH_3CN as elution buffers. The purified peptide was analyzed and characterized by LC-MS (1260 Infinity-6120 Quadrupole LC-MS, Agilent) on Kinetex C8 (4.6 × 75 mm, Phenomenex) with 0.05% TFA/H_2O and 0.05% TFA/CH_3CN as eluents. GLP-1 antagonist was found to be of correct molecular weight and ≥ 95% pure as determined by UV absorbance (λ =214 nm) of the HPLC trace. Lyophilized peptide was stored at 4 °C. Peptide's concentration in prepared aliquots was assessed based on UV absorption at λ = 280 nm measured on a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The extinction coefficient for non-conjugated antagonist at λ = 280 nm was calculated using on-line Peptide Property Calculator (Innovagen, PepCalc.com).

2.12. Statistical analysis

Based on results from previous studies with the same animal model from our group [27] and assuming a conservative difference on the effect level of five infusions with a conservative standard deviation of three infusions, and targeting 80% power with 5% type I error, we estimated that seven mice per group were needed to detect a difference between two groups, which were in line with the number of mice used in previous studies. To assess the time-course of intragastric self-feeding, non-linear mixed models were used, in order to account for correlated variability associated with repeated measures taken within the same animal. For each animal, the cumulative number of infusions was calculated for times 10, 20, 30, 40, 50, and 60 min following the initiation of the training session. The model structure was defined as: Cumulative Infusions = Inf_{max}·Time/(Inf_{50} + Time), were Inf_{max} are the maximum number of cumulative infusions and Inf_{50} is the time needed to reach 50% of the maximum cumulative infusions. Number of cumulative infusions were defined as dependent variables, with Inf_{max} and Inf_{50} as random and fixed effect parameters, respectively. Treatment was used as a covariate on Inf_{max} to assess the effect of treatment on the

number of cumulative infusions. In the pooled analysis of experiments, we performed an unadjusted and adjusted analysis to correct for potential systemic deviations between the experiment cycles. Models with and without treatment effect covariate were compared using the log-likelihood criterion. Maximum likelihood was used for model estimation. Additional statistical analysis was performed using two-tailed paired sample *t*-test or one-way or two-way repeated-measures ANOVA. When ANOVA indicated a significant difference among groups, these groups were compared by Tukey's or Fischer's least significant difference (LSD) *post hoc* test. Pearson correlation analysis was used to determine the correlation between the plasma CCK and GLP-1 concentrations and between peptides and fat infusions. All results are expressed as means ± SEM, and 95% confidence intervals (CI). Statistical significance was set at $p < 0.05$. Data analysis was performed using Graphpad Prism (PASW Statistics Release 18.0.0) or R (version 3.5.2, R: a language and environment for statistical computing, Vienna, Austria).

2.13. Chemicals and drugs

Devazepide was purchased from Sigma-Aldrich (Denmark A/S, Denmark) and BIIE 0246 was purchased from Tocris (Bio-Techne Ltd, Abingdon, United Kingdom).

3. Results

3.1. The gastrointestinal system can sense differences in triacylglycerol concentration

For the following experiments, we used a fat self-administration model where mice associate dry licking on a metallic spout with intragastric infusions of fat [27]. To confirm the ability of our mice to behave as previously described, we performed a test where mice trained to self-infuse IL30 through daily one-hour sessions and following a stable number of IL30 infusions over five days, were allowed to self-infuse IL5. The substitution of IL30 with a six-fold diluted emulsion with respect to triacylglycerol is expected to motivate mice to trigger more infusions in order to reach the same amount of triacylglycerol molecules as they ingested with IL30. The mice were able to sense the triacylglycerol concentration difference and compensated for the decreased triacylglycerol concentration by responding with a 3.4-fold increase in their number of infusions (Fig. 1A, C, D). The expected increase in the number of infusions based on the calorie content of the two emulsions is 3.8-fold. Statistical analysis of the number of infusions during the first 10 min of the 1 h sessions revealed a delay in the compensating response to the IL5 emulsion (Fig. 1B). All assays were performed on animals from the same batch of catheter-implanted animals that were able to be trained in the self-administration set-up (n_{total}=10). The sample size differences in each legend are indicating missing data (e.g. animals not-able to achieve a stable baseline prior to treatment or missing plasma samples).

3.2. Blockade of the GLP-1 receptors increases blood glucose levels

Jant4-C16, a GLP-1 receptor antagonist, is a fatty acid acylated peptide with a prolonged half-life in plasma that has been shown to cause an increase in body weight, food intake, and glucose intolerance in diet-induced obese mice [36]. More specifically, when 0.5 μmol kg^{-1} of Jant4-C16 was dosed subcutaneously 24 h prior to GGTT, diet-induced obese mice exhibited significant glucose intolerance, which suggests that the antagonist blocked the physiological incretin effect of endogenous GLP-1 [36]. Based on this information, we started by assessing the effect of Jant4-C16 on the blood glucose levels of lean mice following a glucose tolerance challenge. To ensure adequate exposure of the GLP-1 antagonist, Jant4-C16 was administered at a dose of 1 μmol kg^{-1}, which is two-fold higher than the dose previously tested and dosed at two intervals, 23 h and 5 h prior to the GGTT. The 23 h time point was selected so that the same dose-schedule could be followed during the fat self-administration tests where the mice were trained daily, as a 24 h

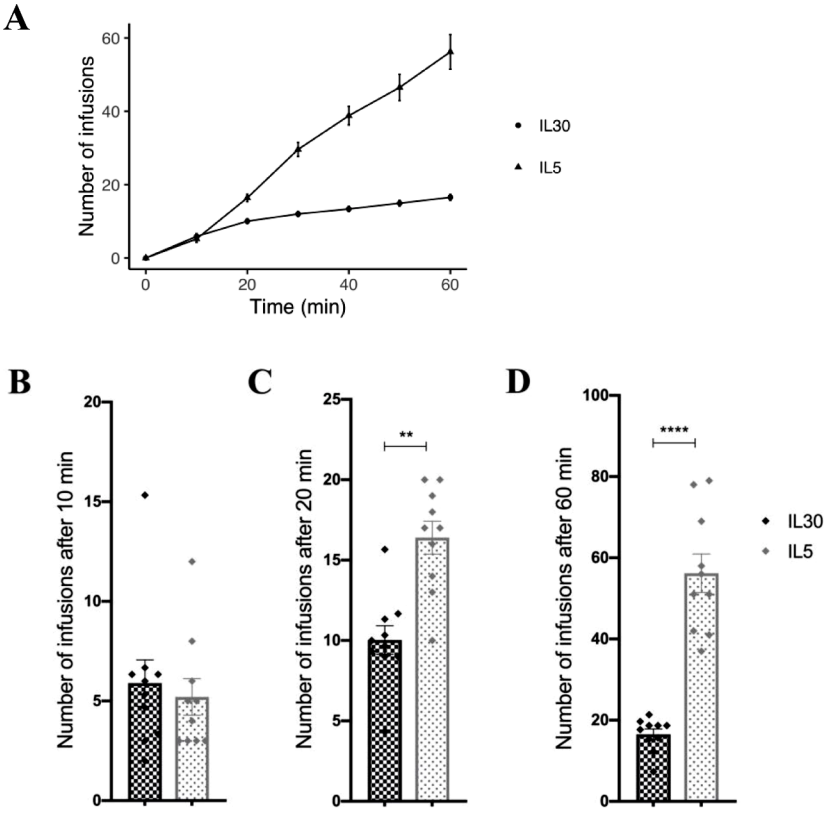

Fig. 1. Mice increase the number of intragastric self-infusions to compensate for lower content of triacylglycerol. Mice (n = 10) were trained to self-infuse IL30 during 1 h sessions. When a stable baseline (five consecutive days of less than 20% variation in the number of IL30 infusions) was achieved, they were presented with IL5. (A) Longitudinal presentation of the number of IL30 and IL5 self-infusions. (B-D) Mice increase their number of self-infusions when switched to IL5 with a delay of 10 min, $^{**}p = 0.0047$, $^{****}p < 0.0001$ in paired sample *t*-test. Data: mean ± *s*.e.m.

dose prior to the training session would coincide with the training session of the previous day. Significant differences in blood glucose levels were observed at the basal state with animals receiving Jant4-C16 having higher blood glucose levels, a difference that remained significant nearly throughout the test with blood glucose levels decreasing at 120 min (Fig. 2). Significant differences were observed between blood glucose levels at baseline and post glucose infusion (vehicle (mmol/L), 2.8 [1.2–4.4]; Jant4-C16 (mmol/L), 3.0 [1.8–4.2]). This finding demonstrates the efficiency of the Jant4-C16 GLP-1 receptor antagonist and suggests a role for endogenous GLP-1 in regulating plasma glucose levels in lean mice.

3.3. Endogenous CCK and GLP-1 regulate post-oral voluntary fat intake

We now raise the question of which gut peptides are important for maintaining a stable calorie intake in the absence of oral cues. To investigate the role of CCK in our mouse model, we employed a specific and well documented CCK_1 receptor antagonist, devazepide. Mice were trained to self-administer IL30 and were dosed with devazepide or vehicle 30 min prior to the 1 h session in a cross-over design. Treatment with devazepide induced a significant increase (34.7%) of IL30 infusions that was more pronounced during the first 30 min of the session (Fig. 3A). No significant differences in intake were observed the first 10 min of the session (Fig. 3E).

Moreover, we hypothesized that GLP-1 might contribute to appetite regulation. Accordingly, we tested a GLP-1 receptor antagonist on the self-administration model in the same manner as with devazepide. Given the long half-life of Jant4-C16 the dose was administered subcutaneously well ahead of the behavior session minimizing the stress-induced variation. Jant4-C16 caused a significant but modest increase (18%) of IL30 infusions (Fig. 3B). Similar to devazepide, no significant differences were observed the first 10 min of the session (Fig. 3E). The above results suggest that both CCK and GLP-1 are involved in the regulation of fat intake in the absence of oral cues.

3.4. Increased post-oral fat intake following combined blockade of CCK_1 and GLP-1 receptors

Given the effects observed by individual blockade of either CCK_1 or GLP-1 receptors, we evaluated the combined blockade of the two types of receptors in the regulation of calorie intake. Jant4-C16 and devazepide were dosed as previously, and the number of IL30 self-infusions was recorded. Interestingly, the mice increased (49%) their number of IL30 infusions to a level that is roughly equal to the combined blockade of each individual peptide action alone (Fig. 3C, Table 2). Thus, in this behavioral set-up, more than one gut-derived peptides seem to be needed in order to regulate fat ingestion with CCK and GLP-1 having an important role. Blood glucose was measured one-hour prior to the session in order to confirm the increased basal glucose levels observed during the GGTT (Fig. 3D). Further analysis of the IL30 self-infusions during the first 10 min showed no significant differences when mice were treated with devazepide or Jant4-C16 while a significant increase in the number of IL30 self-infusions was observed following combined treatment with devazepide and Jant4-C16 at this timepoint (Fig. 3E). We also investigated the role of endogenous PYY in combination with CCK. A Y_2 receptor antagonist was administered in combination with devazepide. No additional effect on the number of IL30 infusions was observed under these conditions (Fig. 4C).

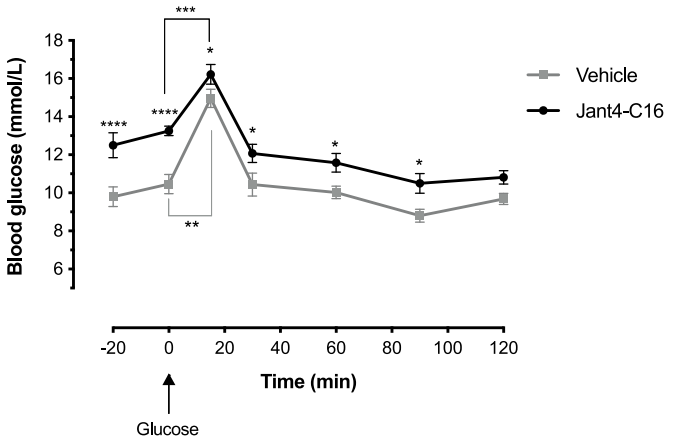

Fig. 2. Jant4-C16 increases blood glucose levels in an oral glucose tolerance test. Blood glucose-time profiles of a glucose tolerance test performed in 6 h fasted male mice ($n = 9$). Blood samples were collected 20 min prior to intragastric acute glucose infusion (2 g kg^{-1}) and over a period of 120 min post-dosing. Mice received either vehicle (gray squares) or 1 μmol kg^{-1} of Jant4-C16 (black circles) 23 h and 5 h in advance. Both treatments were dosed subcutaneously (4 mL kg^{-1}). Data: mean ± s.e.m.; *$p < 0.05$, ****$p < 0.0001$ vs. vehicle control group in two-way repeated-measures ANOVA followed by Fisher's LSD test; ***$p < 0.0005$, **$p < 0.005$ vs post-glucose infusion in two-tailed paired sample *t*-test.

3.5. Plasma CCK and GLP-1 increase during voluntary post-oral fat intake and negatively correlate with the number of infusions at the end of the session

Next, we aimed at confirming that there is an increase of CCK and GLP-1 plasma concentrations during post-oral voluntary fat intake. After obtaining a stable baseline in the number of infusions, blood samples were collected from a group of mice prior to the session and from another group immediately after the session with IL30. Following plasma analysis, we detected an increase in both CCK and GLP-1 plasma concentrations (Fig. 5A and B) while further analysis of the data revealed a positive correlation between CCK and GLP-1 plasma levels (Fig. 5C). Furthermore, correlation analysis between the plasma CCK and GLP-1 levels with IL30 infusions during the last 20 min of the behavior session showed an inverse correlation between peptides and fat intake further supporting a satiation role for prandially released CCK and GLP-1 (Fig. 6A and B). The last 20 min of the 1 h session was analyzed as the number of IL30 self-infusions was reaching a plateau at that time.

4. Discussion

While the role of endogenous CCK in the regulation of food intake is well documented, the role of endogenous GLP-1 and PYY in the regulation of food intake remains unclear [15,17,38,41–43]. Here, we used specific pharmacological antagonists for the CCK_1, GLP-1, and Y_2 receptors to investigate the role of these peptides in regulating fat-induced satiation. Our results, obtained with a behavioral mouse model of intragastric fat self-administration indicate that endogenous CCK and GLP-1 are required in the regulation of post-oral fat intake and that CCK and GLP-1 plasma levels increase during voluntary post-oral fat intake while correlating inversely with the terminal fat intake. Our data confirm that endogenous CCK is a strong regulator of acute fat intake and suggest that endogenous GLP-1 and CCK act additively to regulate post-oral fat intake.

Studies with inhibitors of pancreatic lipase, which is an enzyme active in the intestinal lumen, have shown that fat digestion into fatty acids and 2-MAG is a crucial step for absorption as well as termination of intake [44,45]. Different sensing mechanisms for free fatty acids have been proposed [46,47]. Free fatty acids are ligands for the FFA1 and FFA4 receptors, which are reported to mediate both CCK and GLP-1 secretion, with conflicting results about the involvement of FFA4 in GLP-1 secretion [35,48–51]. Intracellular glycolysis has also been shown to be implicated in fatty acid-induced release of GLP-1 in immortalized enteroendocrine GLUTag cells [52]. In contrast, so far, there is only one known 2-MAG receptor, the GPR119 receptor, which is also involved in

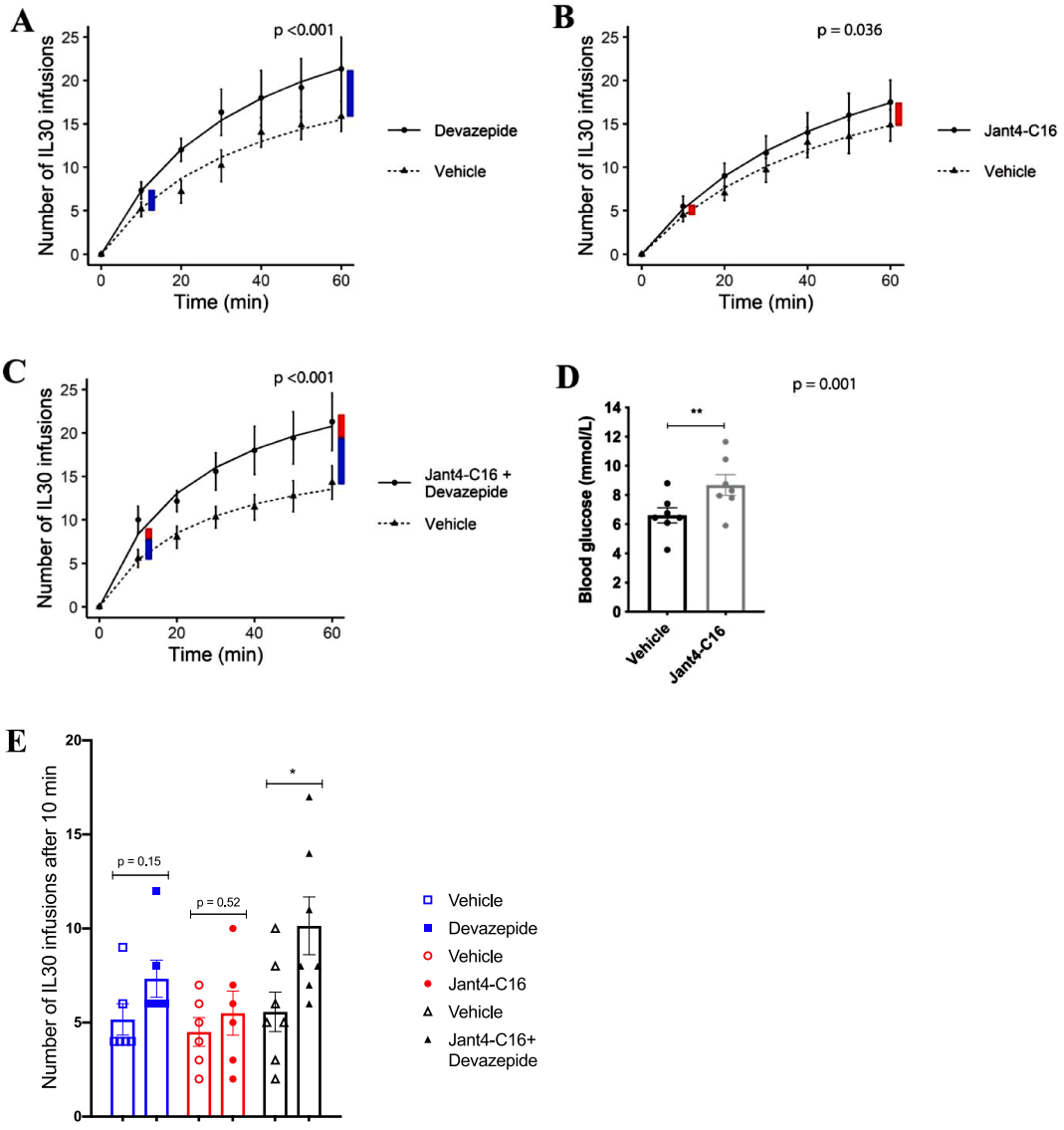

Fig. 3. Blockade of the CCK_1 and GLP-1 receptors increases the number of IL30 intragastric self-infusions. Mice were trained to self-infuse IL30 during 1 h sessions. When the mice achieved a baseline of maximum 20% variation between five consecutive days, they were treated with one of the two receptor antagonists. (A) Devazepide (2 mg 10 mL^{-1} kg^{-1}, black line) or vehicle (10 mL kg^{-1}, dotted line) were dosed intraperitoneally in a cross-over design ($n = 7$). The devazepide treatment significantly increased the number of infusions (non-linear mixed-effects model, $p < 0.001$). (B) Jant4-C16 (1 μmol 4 mL^{-1} kg^{-1}, black line) or vehicle (4 mL kg^{-1}, dotted line) were dosed subcutaneously in a cross-over design ($n = 6$). The Jant4-C16 treatment significantly increased the number of infusions (non-linear mixed-effects model, $p < 0.036$). (C) Mice were treated with Jant4-C16 (1 μmol 4 mL^{-1} kg^{-1}) and devazepide (2 mg 10 mL^{-1} kg^{-1}) or vehicle in a cross-over design ($n = 7$). The combined treatment significantly increased the number of infusions (non-linear mixed-effects model, $p < 0.001$). (A-C) The red and blue bars illustrate the difference in the number of IL30 self-infusions between vehicle and Jant4-C16 and/or devazepide at the first 10 min and at the end of the 1 h session. (D) Mice have increased blood glucose levels prior to the 1 h session with IL30 after being dosed with Jant4-C16. Blood glucose levels were measured after dosing with Jant4-C16 and prior to dosing with devazepide, $^{**}p = 0.001$ in paired sample *t*-test. (E) Analysis of the first 10 min of the 1 h sessions shown in A, B, and C revealed a significant increase in the number of infusions following combined treatment with Jant4-C16 and devazepide. No significant differences were found for Jant4-C16 or devazepide alone. A 7 days wash-out period was allowed in between all different dosings. Data: mean ± *s*.e.m.

GLP-1 secretion [31,34,53]. It has been demonstrated that voluntary ingestion of fat and oral gavage of fat can result in dopamine release in the dorsal striatum and signal reward [24,54]. Kleberg et al. have further compared the effect of fatty acids and 2-MAG in reward signaling and showed that it is the fatty acids that activate dopamine release and not 2-MAG [27]. The notion that there are two different sensing mechanisms for the fat digestion products, which are activated at the same time, led us to hypothesize that endogenous CCK and GLP-1 may act additively or even synergistically to promote satiation.

Furthermore, CCK and more specifically the CCK receptors in the vagus have been shown to mediate satiation, but more interestingly to mediate dopamine release in the brain and to couple in this way food reinforcement and satiation processes [29]. Satiation is usually associated with reduced reinforcement of the ingested food, and self-administration paradigms similar to our intragastric Intralipid self-administration are commonly used to measure reinforcement in addiction research [55]. Pharmacological activation of GLP-1 receptors can in rodents also reduce reinforcement of several substances (e.g., ethanol and amphetamine) [30], but not of opioids [59]. Thus, what we measured in our study could also be called reduced reinforcement of the self-administered Intralipid [29,56,57]. In the context of our study we prefer to call it satiation because (1) we measured the intake of the administered emulsion itself and not its effect on the consumption of concurrently ingested food or liquid as was done in some other studies

Table 2
Unadjusted and adjusted estimates for mixed-effects model analysis predicting the change of cumulative number of infusions for each treatment as compared to the control.

Variables	Unadjusted Analysis			Adjusted Analysis		
	Estimate	95% CI*	p-value	Estimate	95% CI*	p-value
Antagonist (Number of infusions)						
Jant4-C16	2.9	0.2 - 2.9	0.036	4	0.8 - 7.2	0.014
Devazepide	9.7	6.7 - 9.7	<0.001	9.4	6 - 12.7	<0.001
Jant4-C16 + Devazepide	12.5	9.5 - 12.5	<0.001	11.9	8.5 - 15.2	<0.001
Experiment cycle (Number of infusions)						
Jant4-C16				−1.9	−5.4 - 1.7	0.29
Jant4-C16 + Devazepide				0.3	−2.9 - 3.5	0.85

95% CI*: Approximate 95% confidence intervals. Estimates represent difference from control. Adjusted estimates represent difference from Devazepide (reference). Analyses were performed with pooled data from the three experimental cycles (Devazepide, Jant4-C16, and Devazepide+Jant4-C16). Unadjusted analysis was performed using the treatment effect as a covariate only. Adjusted analyses were performed to correct for potential systemic deviations in the experiment cycles using the treatment effect and the experiment cycle as covariates simultaneously.

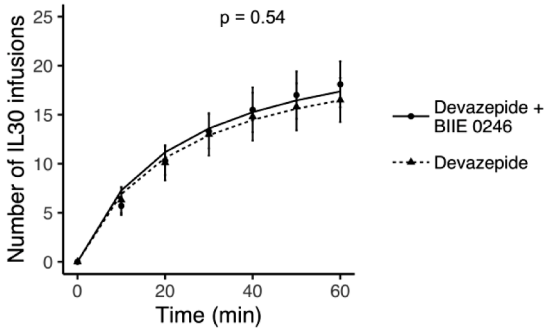

Fig. 4. Simultaneous blockade of CCK_1 and Y_2 receptors did not further increase the number of IL30 intragastric self-infusions. Mice ($n = 7$) were trained to self-infuse IL30 during 1 h sessions. When the mice achieved a baseline of maximum 20% variation between five consecutive days, they were treated with a mix of BIIE 0246 and devazepide (2 mg 2 mg 10 mL^{-1} kg^{-1}) or vehicle in a cross-over design (non-linear mixed-effects model, $p = 0.54$). Data: mean ± *s*.e.m.

(e.g., Lucas and Sclafani, 1999, [58]), (2) we think that the intragastric self-administration of Intralipid is more similar to the ingestion of a meal than to the parenteral self-administration of a drug of abuse, and (3) we functionally relate the observed effects to gut peptides that are well established satiating hormones.

In addition to the CCK receptors, the vagal afferents are known to express GLP-1 receptors [60], but the role of the GLP-1 receptors in the vagus has not been extensively investigated. In a study by Krieger et al. it was shown that reduced vagal expression of the GLP-1 receptor caused enhanced gastric emptying and increased meal size and duration, without affecting total daily intake and body weight [61]. In the same study the knockdown of the vagal afferent GLP-1 receptors blunted the insulin release induced by a meal indicating that also the acute incretin effect of endogenous GLP-1 in response to a meal is in large mediated by GLP-1 receptor expressing vagal afferents [61].

Fat is a potent stimulator of CCK secretion and satiation. CCK is the best described and only well-accepted peptide fulfilling the criteria for an endogenous satiation signal [62,63]. Using devazepide, a CCK_1 receptor antagonist, we demonstrated that endogenous CCK is required for post-oral fat-induced satiation. This is in line with previous studies with CCK_1 receptor antagonists in both rodents and humans [9,64,65]. The role of endogenous GLP-1 in food-induced satiation is not as clear. Exendin (9–39) is a water-soluble GLP-1 receptor antagonist with an estimated elimination half-life of 1.81 h in humans [66] that has been used in both humans and rodents to explore the role of endogenous GLP-1 in food intake with inconsistent results. In rodents, studies have shown that i.p. administration of exendin (9–39) stimulated eating under certain conditions when food intake was normally low [67], when administered centrally in satiated rats [68] or when i.p. administered following a nutrient pre-load [19] while other studies reported that exendin (9–39) even decreased meal duration [16] or had no significant effect on food intake [17,18]. Furthermore, rats fed standard chow or high-fat diet while treated chronically with exendin (9–39) did not change their caloric intake [15]. Surprisingly, exendin (9–39) reduced high-fat diet-induced body weight gain by increasing energy expenditure [15]. In contrast, in mice, GLP-1 receptor antagonism prevented high-fat diet-induced body weight gain by decreasing food intake [41]. The results obtained from such experiments with receptor antagonists are dependent on the pharmacodynamic and pharmacokinetic properties of the antagonist, thus the method and site of administration as well as the dose selection may explain the reported differences to some extent.

It seems that in rodents, the effect on food intake observed after blockade of the GLP-1 receptor depends on the nutritional and metabolic state of the animal. This is further supported by a study by Ronveaux et al. where they showed that the localization of the GLP-1 receptors is changed by feeding, with the receptors being trafficked to the cell membrane of the vagal afferents 40 min following rat refeeding after an overnight fasting [10]. It is possible that GLP-1 requires the secretion of another gut peptide such as CCK in order to elicit its effect on intake regulation. Two human studies exist where the effect of endogenous GLP-1 in food intake was studied. In both studies, participants were infused with exendin (9–39) and received a pre-meal prior to measuring ad libitum food intake. No significant difference in energy intake was observed in either study [21,22].

In our fat self-administration model, the experimental design did not allow an oral pre-load as this would reduce the animal's motivation to learn to associate dry licking with intragastric fat loads. Nevertheless, blockade of the GLP-1 receptors with Jant4-C16 induced a small but significant increase in voluntary post-oral fat intake despite the increased glucose blood levels observed. In view of the fact that hyperglycemia can suppress food intake in rodents [69,70], it is possible that the effect of GLP-1 was partially masked by the elevated blood glucose and a euglycemic clamp might have been beneficial. Interestingly, when mice were dosed with both devazepide and Jant4-C16, they readily increased their number of IL30 self-infusions within 10 min into the session despite their increased plasma glucose levels. This rapid increase in self-infusions was not observed when the mice were switched from IL30 to the diluted emulsion, IL5, which is suggestive of pre-absorptive non-oral related processes taking effect when the CCK_1 and GLP-1 receptors were blocked. In rats, little fat was absorbed within 30 min following intestinal infusion [71,72], thus, the 10 min delay observed after switching IL30 to IL5 may be because the self-infused fat must be digested to fatty acids and 2-MAG before it is detected by intestinal sensors. Thus, the observed increase in IL30 self-infusions following administration of the CCK_1 and GLP-1 receptor antagonists during the first 10 min of the session maybe a result evoked by the basal secretion of CCK and GLP-1 since both peptides are tonically secreted into the circulation during fasting conditions [73]. Endogenous GLP-1 has been previously shown to regulate plasma glucose under fasting conditions in Phox2b specific GLP-1 receptor knockout mice, which lack

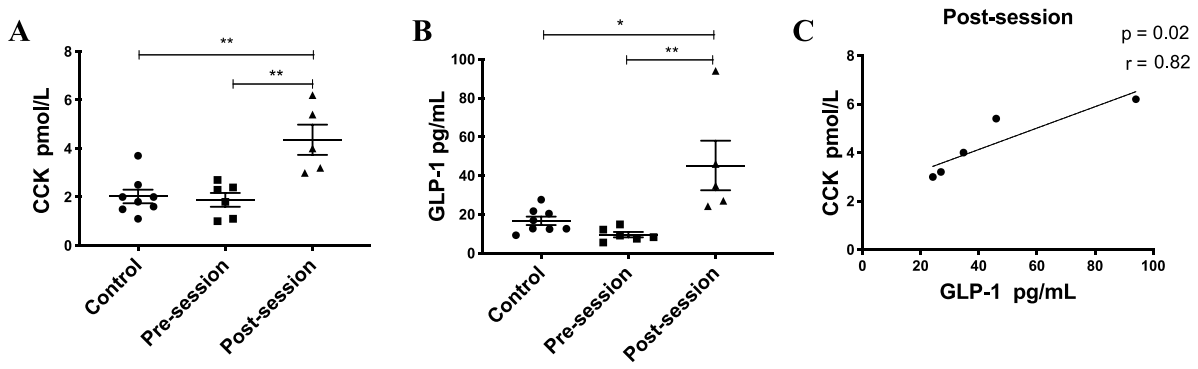

Fig. 5. Plasma GLP-1 and CCK increase following voluntary intragastric self-administration of IL30. Mice (n = 5–8) were trained to self-administer IL30 during 1 h sessions. When the mice achieved a baseline of maximum 20% variation between five consecutive days, blood samples were collected either before or after the IL30 session. Blood was also drawn from mice that were naïve to the self-infusion training. (A) Voluntary IL30 infusion increased plasma CCK levels, $^{**}p$ = 0.0016. (B) GLP-1 plasma levels were significantly increased compared to both pre-session and naïve-control mice, $^{*}p$ = 0.0109, $^{**}p$ = 0.0032. Analysis of data was performed via a one-way ANOVA analysis with a Tukey's multiple comparisons test. (C) Linear fit displaying the relation between plasma levels of CCK and GLP-1 reveals an association between the levels of the two peptides. Analysis of data was performed via Pearson correlation coefficients (n = 5).

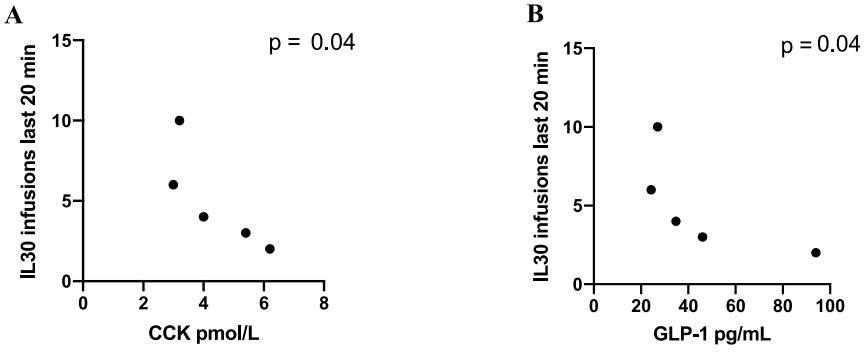

Fig. 6. Post-session plasma levels of CCK and GLP-1 correlate inversely with IL30 self-infusions during the last 20 min of the one-hour session. Mice (n = 5) were trained to self-administer IL30 during 1 h sessions. When the mice achieved a baseline of maximum 20% variation between five consecutive days, blood samples were collected either before or after the IL30 session. Blood was also drawn from mice that were naïve to the self-infusion training. (A) CCK levels correlated inversely with intake during the last 20 min. (B) GLP-1 level correlated inversely intake during the last 20 min. Analysis of data was performed via Pearson correlation coefficients.

the expression of the GLP-1 receptor in both the nodose ganglia and the dorsal motor nucleus of the vagus [74]. Interestingly no differences in fasting blood glucose or blood glucose during an OGTT were observed following knock down of the vagal afferent GLP-1 receptors in rats [61], possibly suggesting that Jant4-C16 may have reached the GLP-1 receptors expressed in the dorsal motor nucleus of the vagus, which have been implicated in the pancreatic vago-vagal reflex and insulin secretion [75–77]. Our results also support a regulatory role for basally secreted GLP-1 on plasma glucose and further suggest that pre-prandial GLP-1 levels together with CCK regulate fat intake. The additive effect of devazepide and Jant4-C16 on fat intake is supported by reported weight reduction in diet-induced obese mice by a GLP-1:CCK fusion peptide [78].

In the present study, mice were calorie-restricted to 75% of normal chow intake during the behavior experiments, and they partly compensated via the Intralipid self-infusions to a level of approximately 90% of the energy intake of control mice. The diet of our self-administration mice consisted of approximately 28% fat in total energy intake when calculated based on the sum of the fat intake during the one-hour self-administration session and the fat content of the pre-weighted pellet given following the session. This diet is not particularly high in fat, and this fat intake seems not to hinder the anorectic effect of endogenous GLP-1 [79]. Our results are also in line with previous observations where increased fat intake alone did not affect GLP-1 secretion in the lymph when not accompanied by increased energy intake [80,81]. The self-administration sessions were running during the light phase when mice typically have a lower food intake. Even though mice are nocturnal animals, it has been shown that their food patterns and activity during the light-phase can be changed when they are food-restricted and entrained to a regular daily mealtime [82]. We have observed that mice consume 0.5 (± 0.4) g of chow (i.e. 16.3% of total energy intake) during one hour after the session, confirming an increased eating activity during the light phase.

Fat ingestion stimulates PYY secretion, and as with GLP-1 and CCK, the secretion is dependent on fat hydrolysis [44,83]. In the mouse, PYY is co-expressed with GLP-1 in the enteroendocrine cells, with the number of secreting cells increasing distally in the small intestine, and with the highest numbers detected in the colon [84,85]. In the mouse colon, agonism of the FFA1, FFA4, and GPR119 receptors generates PYY mediated inhibitory responses, which is suggestive of PYY secretion in response to FFA1, FFA4, or GPR119 receptor activation [86,87]. In response to food intake, enteroendocrine cells secrete PYY(1–36), which is rapidly cleaved into PYY(3–36) by DPP4 [88]. Administration of exogenous PYY(3–36) has been repeatedly shown to decrease food intake in both humans and rodents [89–92], while PYY(1–36) failed to suppress food intake in DPP4-deficient mice [93]. The anorectic effect of PYY(3–36) is thought to be mediated by the Y_2 receptor as blockade of the receptor with the selective antagonist BIIE 0246 as well as deletion of the Y_2 receptor gene in mice abolishes the suppressive effect of exogenous PYY(3–36) on food intake [38,39,89,90]. Administration of BIIE 0246 alone failed to increase food intake in two studies [38,39] but attenuated the anorectic effect of a smaller but not larger, intragastric load of fat in rats [38]. Given the lack of effect of Y_2 antagonism on voluntary food intake in previous studies, we aimed to investigate whether endogenous PYY coacts with endogenous CCK to suppress fat intake. When the fat self-administration mice were challenged with both devazepide and BIIE 0246, no additional increase in the number of voluntary fat self-infusions was observed, indicating an apparent lack of additive effect of endogenous PYY on fat intake in the present experimental design. Future work is needed to explore the extend at which Y_2

receptor antagonism alone induces changes in post-oral fat intake, as well as to confirm the increase in PYY plasma levels [88].

The post-session plasma levels of CCK and GLP-1, which were positively correlated, support the anorectic actions of endogenous CCK and GLP-1, as seen by the inverse correlation between these plasma levels and the fat intake during the last 20 min of the one-hour self-administration of fat. It is clear from the time course of the self-administration that the intake was initially high and leveling out within the last 20 min. This can be explained by a high initial fat intake, which stimulated the release of satiation hormones like CCK and GLP-1. The observed inhibition of satiation by Jant4-C16 may be mediated by blocking GLP-1 receptors in the brain or on the vagus. However, the inverse correlation between plasma GLP-1 levels and intake during the last 20 min of the session suggests that it is intestinal-derived GLP-1, which activated peripherally expressed GLP-1 receptors possibly on the vagus in this animal model. The high volume of liquid intake leading to possible distension of the stomach may not have an important anorectic function in this animal model, since presenting mice with IL5 resulted in a threefold increase in the number of self-infusions and consequently a threefold volume increase (probably due to lack of satiation by nutrient-stimulated hormone release).

The relative effectiveness of each macronutrient in inducing satiation remains unresolved [94–96]. Interestingly, in a recent study in rats, where the satiating effectiveness of isocaloric gastric loads of fat, protein or carbohydrate was evaluated, a similar effectiveness was found across all three macronutrients [94]. These studies are commonly performed with a pre-load of the studied dietary macronutrient where its satiating effectiveness is then assessed on the subsequent energy intake. In contrast to standard experimental designs, the self-administration mouse model allowed us to measure the actual post-oral intake of the studied macronutrient, i.e. fat. Thus, this mouse model allows to investigate specifically the post-oral satiating mechanisms of ingested fat free from confounding factors that derive from orosensory cues and food stuff composed of different dietary macronutrients. Future studies are needed to investigate potential differences between post-oral and oral fat intake in response to the receptor antagonists used in this study.

A limitation of our study is that, due to the time-consuming nature of our behavioral experiments as well as the requirement of maximum two researchers performing the experiments to limit the biological variation, we did not explore the dose-response relationships of the selected antagonists, meaning that it is most likely that the doses used did not elicit the maximum possible pharmacological effect. Devazepide and BIIE 0246 are relatively well explored, thus the doses were selected from prior experimentation in rodents, where their effects on food intake increase was confirmed [39,97,98]. Jant4-C16 has previously been administered i.p. in diet-induced obese mice at the doses of 0.5 μmol/kg and 2 μmol/kg in combination with GLP-1 and s.c. at the dose of 0.5 μmol/kg alone (i.e. with no GLP-1 co-administration) [36]. The authors demonstrated the prolonged pharmacokinetic profile of Jant4-C16 indirectly, via the presence of a glucose intolerance effect 24 h following administration. Here, we used the same indirect approach to ensure that adequate pharmacological effect would be present at the time of the behavioral experimentation. During our pilot studies, and because our preferred route of administration was s.c., we used the previously explored dose of 0.5 μmol/kg, administered 23 h prior to GGTT in our lean mice. Because we observed little to no glucose intolerance effects, we evaluated an increased dose of 1 μmol/kg, which indeed led to glucose intolerance, which however did not reach the previously reported levels (data not shown). Subsequently, we tested the dose of 1 μmol/kg administered 23 h and 5 h prior to GGTT, which led to the establishment of adequate glucose intolerance, thus indirectly confirming that there was adequate Jant4-C16 exposure at the time that the behavioral experiments were designed to take place. The chosen dosing schedule i.e. 23 h and 5 h prior to GGTT, was selected in order to maximize exposure while taking into consideration a possible absorption protraction from the injection site, which has been reported for liraglutide [99]. Thus, a dose 5 h prior to GGTT was deemed more appropriate.

Jant4-C16 was designed based on liraglutide, a GLP-1 receptor agonist with a prolonged half-life [36]. Similar to liraglutide, Jant4-C16 is acylated to palmitic acid and binds to serum albumin with the objective of an extended plasma half-life [36,100]. Even though Jant4-C16 was designed to have a prolonged systemic half-life, its pharmacokinetic properties have not explicitly been evaluated. Thus, an additional limitation of our study is that we did not explore the pharmacokinetic properties of Jant4-C16, meaning that we are unable to report the systemic exposure of the compound. Knowing the pharmacokinetic properties of Jant4-C16, and especially the maximum systemic concentration (C_{max}) and the time that C_{max} is reached (t_{max}) following s.c. administration would be of paramount importance for guiding the dose and dosing regimen prior to the behavioral experimentation. Due to the inherently limiting process of exploring pharmacokinetics in mice, we decided to demonstrate the presence of adequate Jant4-C16 exposure indirectly, via the means of a GGTT, as has previously been proposed [36,41]. However, the glycemic effect observed in this study may be mediated by GLP-1 receptors that are potentially more accessible to Jant4-C16 (such as the pancreatic GLP-1 receptors) as compared to GLP-1 receptors that are involved in food intake regulation, therefore the pharmacodynamics of Jant4-C16 on these receptors may differ.

To conclude, dietary fat induced a release of CCK and GLP-1 in a correlated way and blocking the receptors for these peptides resulted in increased fat-intake compared to blocking each receptor alone. Furthermore, the plasma levels of these peptides were higher the lower the intake was during the last 20 min of the one-hour session further supporting the anorectic action of endogenous CCK and GLP-1 during a voluntary post-oral fat intake.

Funding

This work has been supported by the Lundbeck Foundation to KK, the Augustinus Fonden to HSH, and the Desirée and Niels Yde's Foundation to VV. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

VV, HSH, and KK designed and conceived the study. VV was responsible for the study design, and conduction of the experiments wrote the first draft of the manuscript. MKL, KK, and BL have contributed to the acquisition of the data. VV, HSH, MKL, and TWS have interpreted and analyzed the data. JFR and JHE were responsible for the CCK and GLP-1 analysis, respectively. PAM was responsible for the Jant4-C16 synthesis. All authors contributed to the revising of the manuscript and approved the final version of the manuscript submitted for publication. Correspondence: Vasiliki Vana, Department of Drug Design and Pharmacology University of Copenhagen, Denmark. E-mail: vasiliki.vana@sund.ku.dk

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank Theodoros Papathanasiou for model fitting analysis of the self-infusion data and Quynh Nhu Nguyen for her help during the training of the self-administration model.

References

[1] G.A. Bray, W.E. Heisel, A. Afshin, M.D. Jensen, W.H. Dietz, M. Long, R. F. Kushner, S.R. Daniels, T.A. Wadden, A.G. Tsai, F.B. Hu, J.M. Jakicic, D.

H. Ryan, B.M. Wolfe, T.H. Inge, The science of obesity management: an endocrine society scientific statement, Endocr. Rev. 39 (2018) 79–132, https://doi.org/10.1210/er.2017-00253.
[2] S.J. Guyenet, M.W. Schwartz, Regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity, J. Clin. Endocrinol. Metab. 97 (2012) 745–755, https://doi.org/10.1210/jc.2011-2525.
[3] S. Hu, L. Wang, D. Yang, L. Li, J. Togo, Y. Wu, Q. Liu, B. Li, M. Li, G. Wang, X. Zhang, C. Niu, J. Li, Y. Xu, E. Couper, A. Whittington-Davies, M. Mazidi, L. Luo, S. Wang, A. Douglas, J.R. Speakman, Dietary fat, but not protein or carbohydrate, regulates energy intake and causes adiposity in mice, Cell Metab 28 (2018) 415–431, https://doi.org/10.1016/j.cmet.2018.06.010, e4.
[4] J. Togo, S. Hu, M. Li, C. Niu, J.R. Speakman, Impact of dietary sucrose on adiposity and glucose homeostasis in C57BL/6J mice depends on mode of ingestion: liquid or solid, Mol. Metab. 27 (2019) 22–32, https://doi.org/10.1016/j.molmet.2019.05.010.
[5] A.S. Husted, M. Trauelsen, O. Rudenko, S.A. Hjorth, T.W. Schwartz, GPCR-mediated signaling of metabolites, Cell Metab 25 (2017) 777–796, https://doi.org/10.1016/j.cmet.2017.03.008.
[6] E.K. Williams, R.B. Chang, D.E. Strochlic, B.D. Umans, B.B. Lowell, S.D. Liberles, Sensory neurons that detect stretch and nutrients in the digestive system, Cell 166 (2016) 209–221, https://doi.org/10.1016/j.cell.2016.05.011.
[7] L. Bai, S. Mesgarzadeh, K.S. Ramesh, E.L. Huey, Y. Liu, L.A. Gray, T.J. Aitken, Y. Chen, L.R. Beutler, J.S. Ahn, L. Madisen, H. Zeng, M.A. Krasnow, Z.A. Knight, Genetic identification of vagal sensory neurons that control feeding, Cell 179 (2019) 1129–1143, https://doi.org/10.1016/j.cell.2019.10.031.
[8] D.Y. Kim, G. Heo, M. Kim, H. Kim, J.A. Jin, H.K. Kim, S. Jung, M. An, B.H. Ahn, J. H. Park, H.E. Park, M. Lee, J.W. Lee, G.J. Schwartz, S.Y. Kim, A neural circuit mechanism for mechanosensory feedback control of ingestion, Nature 580 (2020) 376–380, https://doi.org/10.1038/s41586-020-2167-2.
[9] R.C. Ritter, Gastrointestinal mechanisms of satiation for food, Physiol. Behav. 81 (2004) 249–273, https://doi.org/10.1016/j.physbeh.2004.02.012.
[10] C.C. Ronveaux, G. de Lartigue, H.E. Raybould, Ability of GLP-1 to Decrease Food Intake is Dependent on Nutritional Status, Physiol. Behav. 135 (2014) 213–223, https://doi.org/10.1007/978-1-62703-673-3.
[11] R.E. Steinert, C. Beglinger, W. Langhans, Intestinal GLP-1 and satiation: from man to rodents and back, Int. J. Obes. 40 (2016) 198–205, https://doi.org/10.1038/ijo.2015.172.
[12] J.P. Krieger, Intestinal glucagon-like peptide-1 effects on food intake: physiological relevance and emerging mechanisms, Peptides 131 (2020), 170342, https://doi.org/10.1016/j.peptides.2020.170342.
[13] A. Secher, J. Jelsing, A.F. Baquero, J. Hecksher-Sørensen, M.A. Cowley, L. S. Dalbøge, G. Hansen, K.L. Grove, C. Pyke, K. Raun, L. Schäfer, M. Tang-Christensen, S. Verma, B.M. Witgen, N. Vrang, L.B. Knudsen, The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss, J. Clin. Invest. 124 (2014) 4473–4488, https://doi.org/10.1172/JCI75276.
[14] E.B. Rüttimann, M. Arnold, J.J. Hillebrand, N. Geary, W. Langhans, Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms, Endocrinology 150 (2009) 1174–1181, https://doi.org/10.1210/en.2008-1221.
[15] J.-.P. Krieger, W. Langhans, S.J. Lee, Novel role of GLP-1 receptor signaling in energy expenditure during chronic high fat diet feeding in rats, Physiol. Behav. 192 (2018) 194–199, https://doi.org/10.1016/j.physbeh.2018.03.037.
[16] E.B. Rüttimann, M. Arnold, N. Geary, W. Langhans, GLP-1 antagonism with exendin (9-39) fails to increase spontaneous meal size in rats, Physiol. Behav. 100 (2010) 291–296, https://doi.org/10.1016/j.physbeh.2010.02.022.
[17] M. Punjabi, M. Arnold, E. Rüttimann, M. Graber, N. Geary, G. Pacheco-López, W. Langhans, Circulating Glucagon-like Peptide-1 (GLP-1) inhibits eating in male rats by acting in the hindbrain and without inducing avoidance, Endocrinology 155 (2014) 1690–1699, https://doi.org/10.1210/en.2013-1447.
[18] D. Ripken, N. van der Wielen, J. van der Meulen, T. Schuurman, R.F. Witkamp, H. F.J. Hendriks, S.J. Koopmans, Cholecystokinin regulates satiation independently of the abdominal vagal nerve in a pig model of total subdiaphragmatic vagotomy, Physiol. Behav. 139 (2015) 167–176, https://doi.org/10.1016/j.physbeh.2014.11.031.
[19] F.A. Duca, S. Katebzadeh, M. Covasa, Impaired GLP-1 signaling contributes to reduced sensitivity to duodenal nutrients in obesity-prone rats during high-fat feeding, Obesity 23 (2015) 2260–2268, https://doi.org/10.1002/oby.21231.
[20] C.T. Peters, Y.H. Choi, P.L. Brubaker, G.H. Anderson, A glucagon-like peptide-1 receptor agonist and an antagonist modify macronutrient selection by rats, J. Nutr. 131 (2001) 2164–2170. http://www.ncbi.nlm.nih.gov/pubmed/11481412.
[21] R.E. Steinert, J. Schirra, A.C. Meyer-gerspach, P. Kienle, H. Fischer, F. Schulte, B. Goeke, C. Beglinger, Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men, Am. J. Clin. Nutr. 100 (2014) 514–523, https://doi.org/10.3945/ajcn.114.083246.1.
[22] S.J. Melhorn, V. Tyagi, A. Smeraglio, C.L. Roth, E.A. Schur, Initial evidence that GLP-1 receptor blockade fails to suppress postprandial satiety or promote food intake in humans, Appetite 82 (2014) 85–90, https://doi.org/10.1016/j.appet.2014.07.009.
[23] H.S. Hansen, V. Vana, Non-endocannabinoid N-acylethanolamines and 2-mono-acylglycerols in the intestine, Br. J. Pharmacol. 176 (2019) 1443–1454, https://doi.org/10.1111/bph.14175.
[24] J.G. Ferreira, L.A. Tellez, X. Ren, C.W. Yeckel, I.E. de Araujo, Regulation of fat intake in the absence of flavour signalling, J. Physiol. 590 (2012) 953–972, https://doi.org/10.1113/jphysiol.2011.218289.
[25] D.P. Begg, S.C. Woods, Hedonic and homeostatic overlap following fat ingestion, Cell Metab 18 (2013) 459–460, https://doi.org/10.1016/j.cmet.2013.09.012.
[26] M.K. Hankir, F. Seyfried, C.A. Hintschich, T.-.A. Diep, K. Kleberg, M. Kranz, W. Deuther-Conrad, L.A. Tellez, M. Rullmann, M. Patt, J. Teichert, S. Hesse, O. Sabri, P. Brust, H.S. Hansen, I.E. de Araujo, U. Krügel, W.K. Fenske, Gastric bypass surgery recruits a Gut PPAR-α-striatal D1R pathway to reduce fat appetite in obese rats, Cell Metab 25 (2017) 335–344, https://doi.org/10.1016/j.cmet.2016.12.006.
[27] K. Kleberg, A.K. Jacobsen, J.G. Ferreira, J.A. Windeløv, J.F. Rehfeld, J.J. Holst, I. E. de Araujo, H.S. Hansen, Sensing of triacylglycerol in the gut: different mechanisms for fatty acids and 2-monoacylglycerol, J. Physiol. 593 (2015) 2097–2109, https://doi.org/10.1113/jphysiol.2014.285635.
[28] K. Ackroff, A. Sclafani, Post-oral fat stimulation of intake and conditioned flavor preference in C57BL/6J mice: a concentration-response study, Physiol. Behav. 129 (2014) 64–72, https://doi.org/10.1016/j.physbeh.2014.02.047.
[29] W. Han, L.A. Tellez, M.H. Perkins, I.O. Perez, T. Qu, J. Ferreira, T.L. Ferreira, D. Quinn, Z.-.W. Liu, X.-.B. Gao, M.M. Kaelberer, D.V. Bohórquez, S.J. Shammah-Lagnado, G. de Lartigue, I.E. de Araujo, A Neural Circuit for Gut-Induced Reward, Cell 175 (2018) 1–14, https://doi.org/10.1016/j.cell.2018.08.049.
[30] S. Sirohi, J.D. Schurdak, R.J. Seeley, S.C. Benoit, J.F. Davis, Central & peripheral glucagon-like peptide-1 receptor signaling differentially regulate addictive behaviors, Physiol. Behav. 161 (2016) 140–144, https://doi.org/10.1016/j.physbeh.2016.04.013.
[31] K.B. Hansen, M.M. Rosenkilde, F.K. Knop, N. Wellner, T.A. Diep, J.F. Rehfeld, U. B. Andersen, J.J. Holst, H.S. Hansen, 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans, J. Clin. Endocrinol. Metab. 96 (2011) 1409–1417, https://doi.org/10.1210/jc.2011-0647.
[32] H.A. Hassing, M.S. Engelstoft, R.M. Sichlau, A.N. Madsen, J.F. Rehfeld, J. Pedersen, R.M. Jones, J.J. Holst, T.W. Schwartz, M.M. Rosenkilde, H. Hansen, Oral 2-oleyl glyceryl ether improves glucose tolerance in mice through the GPR119 receptor, BioFactors 42 (2016) 665–673, https://doi.org/10.1002/biof.1303.
[33] C.E. Moss, L.L. Glass, E. Diakogiannaki, R. Pais, C. Lenaghan, D.M. Smith, M. Wedin, M. Bohlooly-Y, F.M. Gribble, F. Reimann, Lipid derivatives activate GPR119 and trigger GLP-1 secretion in primary murine ι-cells, Peptides 77 (2016) 16–20, https://doi.org/10.1016/j.peptides.2015.06.012.
[34] M.J. Mandøe, K.B. Hansen, B. Hartmann, J.F. Rehfeld, J.J. Holst, H.S. Hansen, The 2-monoacylglycerol moiety of dietary fat appears to be responsible for the fat-induced release of GLP-1 in humans, Am. J. Clin. Nutr. 102 (2015) 548–555, https://doi.org/10.3945/ajcn.115.106799.
[35] J.H. Ekberg, M. Hauge, L.V. Kristensen, A.N. Madsen, M.S. Engelstoft, A. Husted, R. Sichlau, K.L. Egerod, P. Timshel, T.J. Kowalski, F.M. Gribble, F. Reiman, H. S. Hansen, A.D. Howard, B. Holst, T.W. Schwartz, GPR119, a major enteroendocrine sensor of dietary triglyceride metabolites coacting in synergy with FFA1 (GPR40), Endocrinology 157 (2016) 4561–4569, https://doi.org/10.1210/en.2016-1334.
[36] J.T. Patterson, N. Ottaway, V.M. Gelfanov, D.L. Smiley, D. Perez-tilve, T. Pfluger, M.H. Tsch, R.D. DiMarchi, A novel human-based receptor antagonist of sustained action reveals body weight control by endogenous GLP-1, ACS Chem. Biol. 6 (2011) 135–145.
[37] I. Elmadfa, M. Kornsteiner, Dietary fat intake - A global perspective, Ann. Nutr. Metab. 54 (2009) 8–14, https://doi.org/10.1159/000220822.
[38] R. Reidelberger, A. Haver, P.K. Chelikani, Role of peptide YY(3–36) in the satiety produced by gastric delivery of macronutrients in rats, Am. J. Physiol. Endocrinol. Metab. 304 (2013) E944–E950, https://doi.org/10.1152/ajpendo.00075.2013.
[39] T. Talsania, Y. Anini, S. Siu, D.J. Drucker, P.L. Brubaker, Peripheral exendin-4 and peptide YY3-36 synergistically reduce food intake through different mechanisms in mice, Endocrinology 146 (2005) 3748–3756, https://doi.org/10.1210/en.2005-0473.
[40] J.F. Rehfeld, Accurate measurement of cholecystokinin in plasma, Clin. Chem. 44 (1998) 991–1001, https://doi.org/10.1136/jcp.s1-8.1.26.
[41] B. Svendsen, M.E. Capozzi, J. Nui, S.A. Hannou, B. Finan, J. Naylor, P. Ravn, D. A. D'Alessio, J.E. Campbell, Pharmacological antagonism of the incretin system protects against diet-induced obesity, Mol. Metab 32 (2020) 44–55, https://doi.org/10.1016/j.molmet.2019.11.018.
[42] J.J. Lim, S.D. Poppitt, How satiating are the 'satiety' peptides: a problem of pharmacology versus physiology in the development of novel foods for regulation of food intake, Nutrients 11 (2019) 1–22, https://doi.org/10.3390/nu11071517.
[43] R.E. Steinert, C. Feinle-Bisset, L. Asarian, M. Horowitz, C. Beglinger, N. Geary, C. C.K. Ghrelin, GLP-1, and PYY(3–36): secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB, Physiol. Rev 97 (2017) 411–463, https://doi.org/10.1152/physrev.00031.2014.
[44] M. Ellrichmann, M. Kapelle, P.R. Ritter, J.J. Holst, K.H. Herzig, W.E. Schmidt, F. Schmitz, J.J. Meier, Orlistat inhibition of intestinal lipase acutely increases appetite and attenuates postprandial glucagon-like peptide-1-(7-36)-amide-1, cholecystokinin, and peptide YY concentrations, J. Clin. Endocrinol. Metab. 93 (2008) 3995–3998, https://doi.org/10.1210/jc.2008-0924.
[45] C. Feinle, D. O'Donovan, S. Doran, J.M. Andrews, J. Wishart, I. Chapman, M. Horowitz, Effects of fat digestion on appetite, APD motility, and gut hormones in response to duodenal fat infusion in humans, Am. J. Physiol. Liver Physiol. 284 (2003) G798–G807, https://doi.org/10.1152/ajpgi.00512.2002.
[46] F.A. Duca, J.T.Y. Yue, Fatty acid sensing in the gut and the hypothalamus: in vivo and in vitro perspectives, Mol. Cell. Endocrinol. 397 (2014) 23–33, https://doi.org/10.1016/j.mce.2014.09.022.

[47] B.A. Rasmussen, D.M. Breen, T.K.T. Lam, Lipid sensing in the gut, brain and liver, Trends Endocrinol. Metab. 23 (2012) 49–55, https://doi.org/10.1016/j.tem.2011.11.001.

[48] A. Sankoda, N. Harada, K. Iwasaki, S. Yamane, Y. Murata, K. Shibue, Y. Thewjitcharoen, K. Suzuki, T. Harada, Y. Kanemaru, S. Shimazu-kuwahara, A. Hirasawa, N. Inagaki, Long-chain free fatty acid receptor GPR120 mediates oil-induced gip secretion through CCK in male mice, Endocrinology 158 (2017) 1172–1180, https://doi.org/10.1210/en.2017-00090.

[49] A. Hirasawa, K. Tsumaya, T. Awaji, S. Katsuma, T. Adachi, M. Yamada, Y. Sugimoto, S. Miyazaki, G. Tsujimoto, Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120, Nat. Med. 11 (2005) 90–94, https://doi.org/10.1038/nm1168.

[50] S.J. Paulsen, L.K. Larsen, G. Hansen, S. Chelur, P.J. Larsen, N. Vrang, Expression of the fatty acid receptor GPR120 in the gut of diet-induced-obese rats and its role in GLP-1 secretion, PLoS ONE 9 (2014), https://doi.org/10.1371/journal.pone.0088227.

[51] L. Sundström, S. Myhre, M. Sundqvist, A. Ahnmark, W. McCoull, P. Raubo, S. D. Groombridge, M. Polla, A.C. Nyström, L. Kristensson, M. Någård, M.S. Winzell, The acute glucose lowering effect of specific GPR120 activation in mice is mainly driven by glucagon-like peptide 1, PLoS ONE 12 (2017) 1–22, https://doi.org/10.1371/journal.pone.0189060.

[52] R. Clara, W. Langhans, A. Mansouri, Oleic acid stimulates glucagon-like peptide-1 release from enteroendocrine cells by modulating cell respiration and glycolysis, Metab. Clin. Exp. 65 (2016) 8–17, https://doi.org/10.1016/j.metabol.2015.10.003.

[53] H.S. Hansen, M.M. Rosenkilde, J.J. Holst, T.W. Schwartz, GPR119 as a fat sensor, Trends Pharmacol. Sci. 33 (2012) 374–381, https://doi.org/10.1016/j.tips.2012.03.014.

[54] L.A. Tellez, S. Medina, W. Han, J.G. Ferreira, P. Licona-Limon, X. Ren, T.T. Lam, G.J. Schwartz, I.E. de Araujo, A gut lipid messenger links excess dietary fat to dopamine deficiency, Science 341 (2013) 800–802, https://doi.org/10.1126/science.1239275.

[55] T.A. Green, M.T. Bardo, Opposite regulation of conditioned place preference and intravenous drug self-administration in rodent models: motivational and non-motivational examples, Neurosci. Biobehav. Rev. 116 (2020) 89–98, https://doi.org/10.1016/j.neubiorev.2020.06.006.

[56] I.E. de Araujo, M. Schatzker, D.M. Small, Rethinking Food Reward, Annu. Rev. Psychol. 71 (2020) 1–26, https://doi.org/10.1146/annurev-psych-122216-011643.

[57] L.A. Tellez, J.G. Ferreira, S. Medina, B.B. Land, R.J. Dileone, I.E. De Araujo, Flavor-independent maintenance, extinction, and reinstatement of fat self-administration in mice, Biol. Psychiatry. 73 (2013) 851–859, https://doi.org/10.1016/j.biopsych.2013.02.028.

[58] F. Lucas, A. Sclafani, Flavor preferences conditioned by high-fat versus high-carbohydrate diets vary as a function of session length, Physiol. Behav. 66 (1999) 389–395, https://doi.org/10.1016/S0031-9384(98)00274-1.

[59] A.B. Bornebusch, A. Fink-Jensen, G. Wörtwein, R.J. Seeley, M. Thomsen, Glucagon-Like Peptide-1 Receptor Agonist Treatment Does Not Reduce Abuse-Related Effects of Opioid Drugs, eNeuro 6 (2019), https://doi.org/10.1523/ENEURO.0443-18.2019.

[60] K.L. Egerod, N. Petersen, P.N. Timshel, J.C. Rekling, Y. Wang, Q. Liu, T. W. Schwartz, L. Gautron, Profiling of G Protein-Coupled Receptors in Vagal Afferents Reveals Novel Gut-to-Brain Sensing Mechanisms, Mol. Metab. 12 (2018) 62–75, https://doi.org/10.1016/j.molmet.2018.03.016.

[61] J.-.P. Krieger, M. Arnold, K.G. Pettersen, P. Lossel, W. Langhans, S.J. Lee, Knockdown of GLP-1 receptors in vagal afferents affects normal food intake and glycemia, Diabetes 65 (2016) 34–43, https://doi.org/10.2337/db15-0973.

[62] G.J. Dockray, Cholecystokinin, Curr. Opin. Endocrinol. Diabetes Obes. 19 (2012) 8–12, https://doi.org/10.1097/MED.0b013e32834eb77d.

[63] H.E. Raybould, Mechanisms of CCK signaling from gut to brain, Curr. Opin. Pharmacol. 7 (2007) 570–574, https://doi.org/10.1016/j.coph.2007.09.006.

[64] N. Geary, Endocrine controls of eating: CCK, leptin, and ghrelin, Physiol. Behav. 81 (2004) 719–733, https://doi.org/10.1016/j.physbeh.2004.04.013.

[65] M. Covasa, R.C. Ritter, Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin, Peptides 19 (1998) 1407–1415, https://doi.org/10.1016/S0196-9781(98)00096-5.

[66] Y. Zou, S.H. Seeholzer, C.M. Ng, F. Tang, D.D. De León, Population pharmacokinetics of exendin-(9-39) and clinical dose selection in patients with congenital hyperinsulinism, Br. J. Clin. Pharmacol. 84 (2017) 520–532, https://doi.org/10.1111/bcp.13463.

[67] D.L. Williams, D.G. Baskin, M.W. Schwartz, Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety, Endocrinology 150 (2009) 1680–1687, https://doi.org/10.1210/en.2008-1045.

[68] M.D. Turton, D. O'Shea, I. Gunn, S.A. Beak, C.M.B. Edwards, K. Meeran, S.J. Choi, G.M. Taylor, M.M. Heath, P.D. Lambert, J.P.H. Wilding, D.M. Smith, M.A. Ghatei, J. Herbert, S.R. Bloom, A role for glucagon-like peptide-1 in the central regulation of feeding, Nature 379 (1996) 69–72, https://doi.org/10.1038/379069a0.

[69] J.D. Davis, D. Wirtshafter, K.E. Asin, D. Brief, Sustained Intracerebroventricular Infusion of Brain Fuels Reduces Body Weight and Food Intake in Rats, Science 212 (1981) 81–83.

[70] K. Kurata, K. Fujimoto, H. Etou, K. Fukagawa, T. Sakata, D-glucose suppression of eating after intra-third ventricle infusion in rat, Physiol. Behav. 37 (1985) 615–620, https://doi.org/10.1016/0031-9384(86)90295-7.

[71] S.M. Yoder, Q. Yang, T.L. Kindel, P. Tso, Stimulation of incretin secretion by dietary lipid: is it dose dependent? Am. J. Physiol. - Gastrointest. Liver Physiol. 297 (2009) 299–305, https://doi.org/10.1152/ajpgi.90601.2008.

[72] D. Greenberg, R.A. Kava, D.R. Lewis, M.R.C. Greenwood, G.P. Smith, Time course for entry of intestinally infused lipids into blood of rats, Am. J. Physiol. - Regul. Integr. Comp. Physiol. (1995) 269, https://doi.org/10.1152/ajpregu.1995.269.2.r432.

[73] M.B. Toft-Nielsen, S. Madsbad, J.J. Holst, The effect of glucagon-like peptide I (GLP-I) on glucose elimination in healthy subjects depends on the pancreatic glucoregulatory hormones, Diabetes 45 (1996) 552–556, https://doi.org/10.2337/diabetes.45.5.552.

[74] E.M. Varin, E.E. Mulvihill, L.L. Baggio, J.A. Koehler, X. Cao, R.J. Seeley, D. J. Drucker, Distinct neural sites of GLP-1R expression mediate physiological versus pharmacological control of incretin action, Cell Rep 27 (2019) 3371–3384, https://doi.org/10.1016/j.celrep.2019.05.055, e3.

[75] S. Wan, F.H. Coleman, R.A. Travagli, Glucagon-like peptide-1 excites pancreas-projecting preganglionic vagal motoneurons, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (2007) G1474–G1482, https://doi.org/10.1152/ajpgi.00562.2006.

[76] T. Babic, K.N. Browning, Y. Kawaguchi, X. Tang, R.A. Travagli, Pancreatic insulin and exocrine secretion are under the modulatory control of distinct subpopulations of vagal motoneurones in the rat, J. Physiol. 590 (2012) 3611–3622, https://doi.org/10.1113/jphysiol.2012.234955.

[77] B.M. Mussa, A.J.M. Verberne, The dorsal motor nucleus of the vagus and regulation of pancreatic secretory function, Exp. Physiol. 98 (2013) 25–37, https://doi.org/10.1113/expphysiol.2012.066472.

[78] D.C. Hornigold, E. Roth, V. Howard, S. Will, S. Oldham, M.P. Coghlan, C. Blouet, J.L. Trevaskis, A GLP-1:CCK fusion peptide harnesses the synergistic effects on metabolism of CCK-1 and GLP-1 receptor agonism in mice, Appetite 127 (2018) 334–340, https://doi.org/10.1016/j.appet.2018.05.131.

[79] P. Richards, R. Pais, A.M. Habib, C.A. Brighton, G.S.H. Yeo, F. Reimann, F. M. Gribble, High fat diet impairs the function of glucagon-like peptide-1 producing L-cells, Peptides 77 (2016) 21–27, https://doi.org/10.1016/j.peptides.2015.06.006.

[80] F. Wang, S.M. Yoder, Q. Yang, A.B. Kohan, T.L. Kindel, J. Wang, P. Tso, Chronic high-fat feeding increases GIP and GLP-1 secretion without altering body weight, Am. J. Physiol. Liver Physiol. 309 (2015) G807–G815, https://doi.org/10.1152/ajpgi.00351.2013.

[81] M. Arnold, Y. Dai, P. Tso, W. Langhans, Meal-contingent intestinal lymph sampling from awake, unrestrained rats, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 302 (2012) R1365–R1371, https://doi.org/10.1152/ajpregu.00497.2011.

[82] G. de Lartigue, M. McDougle, Dorsal striatum dopamine oscillations: setting the pace of food anticipatory activity, Acta Physiol 225 (2019) e13152., https://doi.org/10.1111/apha.13152.

[83] L. Degen, J. Drewe, F. Piccoli, K. Gräni, S. Oesch, R. Bunea, M. D'Amato, C. Beglinger, Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 292 (2007) R1391–R1399, https://doi.org/10.1152/ajpregu.00734.2006.

[84] N.J. Wewer Albrechtsen, R.E. Kuhre, S. Toräng, J.J. Holst, The intestinal distribution pattern of appetite- and glucose regulatory peptides in mice, rats and pigs, BMC Res. Notes. 9 (2016) 1–6, https://doi.org/10.1186/s13104-016-1872-2.

[85] H.J. Cho, S. Kosari, B. Hunne, B. Callaghan, L.R. Rivera, D.M. Bravo, J.B. Furness, Differences in hormone localisation patterns of K and L type enteroendocrine cells in the mouse and pig small intestine and colon, Cell Tissue Res 359 (2015) 693–698, https://doi.org/10.1007/s00441-014-2033-3.

[86] R. Moodaley, D.M. Smith, I.R. Tough, M. Schindler, H.M. Cox, Agonism of free fatty acid receptors 1 and 4 generates peptide YY-mediated inhibitory responses in mouse colon, Br. J. Pharmacol. 174 (2017) 4508–4522.

[87] I.R. Tough, S. Forbes, H. Herzog, R.M. Jones, T.W. Schwartz, H.M. Cox, Bidirectional GPR119 Agonism requires peptide YY and glucose for activity in mouse and human colon mucosa, Endocrinology 159 (2018) 1704–1717, https://doi.org/10.1210/en.2017-03172.

[88] R. Mentlein, P. Dahms, D. Grandt, R. Krüger, Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV, Regul. Pept. 49 (1993) 133–144, https://doi.org/10.1016/0167-0115(93)90435-B.

[89] V. Scott, N. Kimura, J.A. Stark, S.M. Luckman, Intravenous peptide YY3-36 and Y2 receptor antagonism in the rat: effects on feeding behaviour, J. Neuroendocrinol. 17 (2005) 452–457, https://doi.org/10.1111/j.1365-2826.2005.01330.x.

[90] R.L. Batterham, M.A. Cowley, C.J. Small, H. Herzogk, M.A. Cohen, C.L. Dakin, A. M. Wren, A.E. Brynes, M.A. Ghatei, S.R. Bloom, Gut hormone PYY 3-36 physiologically inhibits food intake, Nature 418 (2002) 728–730.

[91] D.E. Cummings, J. Overduin, Gastrointestinal regulation of food intake, Princ, Mol. Med. 117 (2006) 513–517, https://doi.org/10.1007/978-1-59259-963-9_47.

[92] P.K. Chelikani, A.C. Haver, R.D. Heidelberger, Intravenous infusion of peptide YY (3-36) potently inhibits food intake in rats, Endocrinology 146 (2005) 879–888, https://doi.org/10.1210/en.2004-1138.

[93] S. Unniappan, C.H.S. McIntosh, H.U. Demuth, U. Heiser, R. Wolf, T.J. Kieffer, Effects of dipeptidyl peptidase IV on the satiety actions of peptide YY, Diabetologia 49 (2006) 1915–1923, https://doi.org/10.1007/s00125-006-0310-8.

[94] N. Cassie, R. Anderson, D. Wilson, J.G. Mercer, P. Barrett, Fat, carbohydrate and protein by oral gavage in the rat can be equally effective for satiation, Physiol. Behav. 207 (2019) 41–47, https://doi.org/10.1016/j.physbeh.2019.04.022.

[95] F. Lucas, A. Sclafani, Differential reinforcing and satiating effects of intragastric fat and carbohydrate infusions in rats, Physiol. Behav. 66 (1999) 381–388, https://doi.org/10.1016/S0031-9384(98)00275-3.

[96] G.J. Schwartz, C.F. Salorio, C. Skoglund, T.H. Moran, Gut vagal afferent lesions increase meal size but do not block gastric preload-induced feeding suppression, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 276 (1999) 1623–1629, https://doi.org/10.1152/ajpregu.1999.276.6.r1623.

[97] L.A. Brenner, R.C. Ritter, Type A CCK receptors mediate satiety effects of intestinal nutrients, Pharmacol. Biochem. Behav. 54 (1996) 625–631, https://doi.org/10.1016/0091-3057(95)02210-4.

[98] T. Woltman, D. Castellanos, R. Reidelberger, Role of cholecystokinin in the anorexia produced by duodenal delivery of oleic acid in rats, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 269 (1995) R1420–R1433.

[99] K. Madsen, L.B. Knudsen, H. Agersoe, P.F. Nielsen, H. Thøgersen, M. Wilken, N. L. Johansen, Structure-activity and protraction relationship of long-acting glucagon-like peptide-1 derivatives: importance of fatty acid length, polarity, and bulkiness, J. Med. Chem. 50 (2007) 6126–6132, https://doi.org/10.1021/jm070861j.

[100] L.B. Knudsen, J. Lau, The discovery and development of liraglutide and semaglutide, Front. Endocrinol. 10 (2019), https://doi.org/10.3389/fendo.2019.00155.