High-fat diet changes the temporal profile of GLP-1 receptor-mediated hypophagia in rats

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Submitted 17 December 2012; accepted in final form 22 April 2013

Mul JD, Begg DP, Barrera JG, Li B, Matter EK, D’Alessio DA, Woods SC, Seeley RJ, Sandoval DA. High-fat diet changes the temporal profile of GLP-1 receptor-mediated hypophagia in rats. Am J Physiol Regul Integr Comp Physiol 305: R68–R77, 2013. First published April 24, 2013; doi:10.1152/ajpregu.00588.2012.—Over-consumption of a high-fat diet promotes weight gain that can result in obesity and associated comorbidities, including Type 2 diabetes mellitus. Consumption of a high-fat diet also alters gut-brain communication. Glucagon-like peptide 1 (GLP-1) is an important gastrointestinal signal that modulates both short- and long-term energy balance and is integral in maintenance of glucose homeostasis. In the current study, we investigated whether high-fat diets (40% or 81% kcal from fat) modulated the ability of the GLP-1 receptor (GLP-1r) agonists exendin-4 (Ex4) and liraglutide to reduce food intake and body weight. We observed that rats maintained on high-fat diets had a delayed acute anorexic response to peripheral administration of Ex4 or liraglutide compared with low-fat diet-fed rats (17% kcal from fat). However, once suppression of food intake in response to Ex4 or liraglutide started, the effect persisted for a longer time in the high-fat diet-fed rats compared with low-fat diet-fed rats. In contrast, centrally administered Ex4 suppressed food intake similarly between high-fat diet-fed and low-fat diet-fed rats. Chronic consumption of a high-fat diet did not change the pharmacokinetics of Ex4 and liraglutide but increased expression and decreased hindbrain Glp1r expression. Taken together, these findings demonstrate that dietary composition alters the temporal profile of the anorectic response to exogenous GLP-1r agonists.

Glucagon-like peptide 1; food intake; liraglutide; exendin-4; obesity

OBESITY RESULTS FROM AN IMBALANCE between energy intake and energy expenditure. To maintain energy balance, continuous communication between peripheral tissues and the central nervous system (CNS) is necessary, and a crucial part of this communication is the gut-brain axis (9). The gut-brain axis comprises neural, hormonal, and nutritional signals generated prior to, during, and following caloric intake and that prepare the gastrointestinal (GI) system for caloric influx and mediate downstream responses to control ingestion. The overall result is the initiation of a series of compensatory mechanisms to regulate nutrient disposal and energy expenditure, contributing to the homeostasis of energy balance (45).

Glucagon-like peptide 1 (GLP-1), a product of the preproglucagon gene Gcg (34), is an important gastrointestinal (GI) signal that modulates both short- and long-term energy balance and is integral in maintenance of glucose homeostasis (7). GLP-1 is produced in intestinal L-cells and in neurons of the nucleus of the solitary tract (NTS) (39, 49). Peripheral GLP-1 acts as a gut-derived satiety signal, modulating energy balance on a short-term, meal-to-meal basis, whereas it also interacts with leptin, a known regulator of long-term energy balance (7). Further, NTS neurons are necessary for long-term energy balance, as when the expression of Gcg is down-regulated specifically in the hindbrain using a lentiviral approach, body weight and adiposity are significantly elevated (6). After nutrient ingestion, peripheral GLP-1 is secreted from the intestine, and one of its major functions is to augment glucose-stimulated insulin secretion. This has prompted development of GLP-1 receptor (GLP-1r) agonists as treatments of Type 2 diabetes mellitus (T2D) (41). Endogenous circulating active GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to a shorter, inactive peptide (13, 30, 38), and GLP-1 analogs resistant to DPP-4 have consequently been developed. These include exendin-4 (Ex4), a synthetic long-acting GLP-1r agonist (21, 54) that is injected twice daily to treat T2D in humans, and an acylated albumin-bound GLP-1 analog, liraglutide, which is injected once daily (1, 14, 16). Both Ex4 (Byetta) and liraglutide (Victoza) were approved by the U.S. Food and Drug Administration as pharmaceuticals to improve glycemic control in T2D patients. In rodents, when administered peripherally or centrally, both Ex4 and liraglutide potently inhibit food intake and induce body mass loss (5, 7, 35, 42, 43). In humans, GLP-1r agonists also induce weight loss (4, 11, 19, 41), suggesting that GLP-1r-agonism can also be viewed as a potential weight-loss therapy.

Consumption of a high-fat diet alters gut-brain communication and attenuates subsequent satiation signaling (17, 18, 37). This decreased sensitivity to nutrients could result from changes in activity of the hindbrain, altered expression of Glp1r, or desensitized receptor activation. For example, high-fat diet-fed rodents have decreased plasma active GLP-1 levels (3) and impaired satiety responses to Ex4 (53); however, food intake was monitored only up to 24 h following Ex4 administration (53). Finally, another study reported a longer latency for liraglutide-mediated food intake suppression in high-fat/sucrose-fed diet-induced obese rats vs. Chow-fed, nonobese rats (22). Currently, it is unknown whether consumption of a high-fat diet alters gut-brain communication or reduces satiety signaling, or decreases hindbrain Glp1r expression. However, in rodents, we found that high-fat diet-fed rats have decreased hindbrain Glp1r expression (36) compared with low-fat diet-fed rats (22).

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OBESITY RESULTS FROM AN IMBALANCE between energy intake and energy expenditure. To maintain energy balance, continuous communication between peripheral tissues and the central nervous system (CNS) is necessary, and a crucial part of this communication is the gut-brain axis (9). The gut-brain axis comprises neural, hormonal, and nutritional signals generated prior to, during, and following caloric intake and that prepare the gastrointestinal (GI) system for caloric influx and mediate downstream responses to control ingestion. The overall result is the initiation of a series of compensatory mechanisms to regulate nutrient disposal and energy expenditure, contributing to the homeostasis of energy balance (45).

Glucagon-like peptide 1, food intake, liraglutide, exendin-4, obesity

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Rats, Housing, and Diets

Naive male Long-Evans rats (Harlan Laboratories, Indianapolis, IN) were individually housed and maintained on a 12:12 h light-dark cycle (lights off at 1400) at 25°C and 50–60% humidity in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facilities of the Metabolic Diseases Institute of the University of Cincinnati. All rats had ad libitum access to water and one of the following diets: a low-fat pelleted diet (LFD; LM-485 no. 7012, 3.1 kcal/g, 25%, 58%, and 17% kcal from protein, carbohydrate, and fat, respectively; Harlan Teklad, Madison, WI), a pelleted diet containing 40% fat (MFD; D03082706, 4.54 kcal/g, 15%, 46%, and 40% kcal from protein, carbohydrate, and fat, respectively; Open Source Diets, New Brunswick, NJ), or a paste diet containing 81% fat (HFD; Dyet 100959, 6.04 kcal/g, 14%, 5%, and 81% kcal from protein, carbohydrate, and fat, respectively; Dyets, Bethlehem, PA; also see Table 1). All rats had access to home-cage enrichment (red rat retreat; Bioserve Biotechnologies, Beltsville, MD).

Peptides

Ex4 was obtained from American Peptide (Sunnyvale, CA), and liraglutide was a generous gift from Novo Nordisk (Bagsvaerd, Denmark). Ex4 and liraglutide were dissolved in sterile 0.9% saline and PBS, respectively, and administered intraperitoneally in a volume of 1 ml/kg.

Body Composition

Fat mass and lean mass were measured in conscious rats using an EchoMRI analyzer (Echo Medical Systems, Houston, TX) during week 6 (Ex4 dose-response study), week 13 (Ex4 flat-dose study), and week 16 (liraglutide study).

Cohort 1

Effect of high-fat diet on Ex4- or liraglutide-induced hypophagia. Before the start of the experiment, all rats were handled daily and habituated to intraperitoneal injection of 0.5 ml saline and measurement of food intake throughout the dark phase on three occasions. On the first day of the experiment, after 5 wk of maintenance on their designated diet, all rats were weighed, and food was removed 2 h before the onset of the dark. Ex4 (3, 10, or 33 µg/kg) or saline (1 ml/kg) was injected intraperitoneally 1 h before the start of the dark. At the onset of dark, preweighed food was returned to all rats. Each test session was separated by sevenontost days (experiments performed when rats were 5, 6, and 7 wk on the diet). The same strategy was used in administering a flat dose of Ex4 (15 µg·0.5 ml·rat⁻¹) or saline (0.5 ml) when rats had been on their respective diet for 13 wk, and liraglutide (300 µg/kg) or saline (1 ml/kg) when rats had been on their diet for 16 wk. Food intake was measured 2, 4, 24, 48 h postinjection (Ex4 dose-response study), or 2, 4, 24, 48, 72, and 96 h postinjection (Ex4 flat dose and liraglutide studies). Body mass was measured 24 and 48 h postinjection (Ex4 dose-response study), or 24 h postinjection (Ex4 flat dose and liraglutide studies).

Cohort 2

Third cerebroventricular cannulation. For the third cerebroventricular (IVT) experiment, rats were outfitted with a cannula (Plastics One, Roanoke, VA) directed toward the IVT, and correct placement was confirmed as previously described (8). Briefly, rats were anesthetized with intraperitoneal ketamine (70 mg/kg) and xylazine (6 mg/kg). Rats were shaved and positioned with the skull horizontal in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). After a small hole was drilled through the skull at a position 2.2 mm posterior to bregma, on the midline, the sagittal sinus was displaced laterally, and a stainless-steel guide cannula (Plastics One) was lowered 7.5 mm ventral to the dura. The cannula was fixed with dental acrylic anchored to the skull with four screws, and an obturator was inserted that extended 0.5 mm below the cannula. The surgery was performed using sterile techniques. Five days prior to food intake experiments, correct placement and viability of the cannula were confirmed by behavioral means using 10 ng of ANG II (American Peptides, Sunnyvale, CA) in 1 µl of saline. Rats consuming at least 5 ml of water within 30 min were considered to have a viable cannula.

Effect of high-fat diet on the action of IVT Ex4 to induce anorexia. Rats were maintained on either LFD or HFD for 4 wk before IVT cannulation. Rats were allowed to recover for 1 wk before the start of the experiment. Before the start of the experiment, all rats were handled daily and habituated to IVT injection of 1 µl of saline and measurement of food intake throughout the dark phase on two occasions. On the first day of the experiment, after 6 wk of maintenance on their designated diet, all rats were weighed, and food was removed 2 h before the onset of the dark. Ex4 (0.1 µg/1 µl) or saline (1 µl) was injected IVT 1 h before the start of the dark. At the onset of the dark, preweighed food was returned to all rats. Food intake was measured 2, 4, and 24 h postinjection.

Cohort 3

Effect of high-fat diet on Ex4 pharmacokinetics. On the first day of the experiment, after 11 wk of maintenance on their designated diet, all rats were weighed, and food was removed 2 h before the onset of the dark. Intraperitoneal Ex4 (33 µg) was injected 30 min before dark onset, and at the onset of dark, preweighed food was returned to all rats. Tail-vein blood samples were collected before injection (baseline) and 2, 4, and 24 h after injection. Serum samples were obtained by centrifugation and stored at −80°C until required for assessment of Ex4 using a commercially available EIA kit (Bachem, Torrance, CA).

Tissue isolation. After 24 wk on the designated diets, 3-h fasted rats were killed during the first half of the light phase using CO2 asphyxiation followed by decapitation. The whole hypothalamus, an intestinal sample just proximal of the ileocecal valve (distal ileum), the NTS at the level of the area postrema, and the left inferior nodose ganglion of the vagus nerve were isolated. The hypothalamus and NTS samples were cut symmetrically before freezing. All samples were frozen in liquid nitrogen-cooled isopentane as fast as possible and stored at −80°C until further analyses.

Assessment of Glp1r and Gcg mRNAs. RNA was isolated using Agencourt RNAAdvance tissue kit (Beckman Coulter, Brea, CA), and genomic DNA was eliminated using DNeasy tissue kit (Qiagen, Valencia, CA). RNA quantity and quality were assessed using a NanoVue (GE Healthcare, Piscataway, NJ). cDNA was generated by reverse transcription using iScript (Bio-Rad, Hercules, CA), and diluted in MQ (1:6). cDNA was generated simultaneously for all samples to minimize experimental variations. Glp1r and Gcg were amplified from 1 µg of reverse-transcribed total RNA using TaqMan Gene Expression Master Mix (Applied Biosystems, Carlsbad, CA) with Glp1r and Gcg sense and antisense primers, and a dual-labeled probe (5′FAM, 3′-TAMRA) (Applied Biosystems; assay on demand Rn00562406_m1 and Rn00562293_m1, respectively). Samples were run in duplicate, and mRNA expression was normalized to the L32 housekeeping gene. Calculations were per-
formed by a comparative method (2.0–3.4C), taking the efficiency of the PCR into account (2.0). Quantitative PCR was performed on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems).

**Total GLP-1 protein analysis.** Peptides were extracted from frozen tissue samples by homogenization in acetic acid using a boiling water bath and centrifuged to pellet cellular debris (48). Subsequently, the supernatant was air-dried overnight and dissolved in 1.5 ml MQ water. Total GLP-1 in tissue extracts was measured using a commercially available immunometric assay kit (MesoScale Discovery, Gaithersburg, MD), as instructed by the manufacturer. Total proteins displayed as means ± SE. As MFD and HFD rats were heavier at the onset of the experiment, we also administered Ex4 using a flat dose (15 μg/rat) to exclude any effect of dosing strategy. Similar to the dose-response study, administration of 15 μg of Ex4 demonstrated a delayed onset and prolonged hypophagic effect in MFD and HFD rats (Fig. 2, A–E).

**Data Analysis**

Data for each respective study were analyzed separately and displayed as means ± SE. For all experiments, single comparisons between means were analyzed by unpaired Student’s t-test, whereas multiple comparisons between means were analyzed using one-, two-, or three-way ANOVA, with repeated measures where applicable. If appropriate, post hoc analyses were made using Tukey’s honestly significant difference test with P < 0.05 accepted as statistically different. Analyses were made using Statistica 10 (StatSoft, Tulsa, OK).

**RESULTS**

**Effect of High-Fat Diet on Ex4-Induced Hypophagia**

To study the effect of high-fat diet maintenance on GLP-1r agonist-mediated hypophagia, we opted to use two different high-fat diets (Table 1). After 6 wk on their designated diet, MFD and HFD rats had greater body mass (F2,38 = 7.46, P < 0.05) and fat mass (F2,38 = 16.71, P < 0.05) than LFD rats, with no significant difference between MFD and HFD rats (Fig. 1A). Lean mass was similar among all groups (Fig. 1A). Analysis of food intake during the 0–2- and 2–4-h intervals following Ex4 administration revealed a time × diet × drug interaction (F6,111 = 2.26; P < 0.05). During the 0–2-h interval, LFD rats treated with 10 or 33 μg/kg Ex4 had reduced food intake compared with saline-treated controls (P < 0.05; Fig. 1B). However, food intake at this same dose of Ex4 was significantly higher in MFD and HFD rats during this time interval (Fig. 1B). During the 2–4-h interval, treatment with 10 or 33 μg/kg Ex4 reduced food intake in MFD rats compared with saline-treated controls (P < 0.05; Fig. 1C). Analysis of food intake during the 0–24- and 24–48-h intervals following Ex4 administration revealed a time × diet × drug interaction (F6,111 = 3.05, P < 0.05). Post hoc analysis revealed that during the 0–24-h interval, 10 or 33 μg/kg Ex4 treatment significantly reduced food intake compared with saline-treated controls in all diets (P < 0.05; Fig. 1D). However, during the 24–48-h interval, food intake was no longer significantly reduced in LFD rats at any dose of Ex4 but remained suppressed in 33 μg/kg Ex4-treated MFD and 10 and 33 μg/kg Ex4-treated HFD rats (P < 0.05; Fig. 1E). Analysis of food intake during the 0–48-h interval following Ex4 administration revealed a diet × drug interaction (F6,111 = 3.66; P < 0.05). Food intake was decreased in 33 μg/kg Ex4-treated LFD rats, 10 and 33 μg/kg Ex4-treated MFD rats, and 10 and 33 μg/kg Ex4-treated HFD rats compared with their saline-treated controls (Fig. 1F). The reduction in food intake was significantly greater in the 10 and 33 μg/kg Ex4-treated HFD rats than MFD rats treated with a similar dose (P < 0.05; Fig. 1F).

Ex4 treatment lowered body mass over 24 h following Ex4 administration (main effect of drug, F3,111 = 62.35; P < 0.05), with all Ex4-treated rats losing more mass than saline-treated controls in a dose-dependent manner and independent of diet (P < 0.05; Fig. 1G). Forty-eight hours following Ex4 administration, changes in body mass revealed a diet × drug interaction (F6,111 = 4.08; P < 0.05), with 33 μg/kg Ex4-treated LFDRats, 10 and 33 μg/kg Ex4-treated MFD rats, and 10 and 33 μg/kg Ex4-treated HFD rats having lost more body mass than their respective saline-treated controls (P < 0.05; Fig. 1H). Moreover, the 10- or 33 μg/kg Ex4-treated HFD rats had lost significantly more body mass than LFD rats treated with a similar dose (P < 0.05; Fig. 1H).

In the above-mentioned experiment Ex4 was dosed per body mass. As MFD and HFD rats were heavier at the onset of the experiment, we also administered Ex4 using a flat dose (15 μg/rat) to exclude any effect of dosing strategy. Similar to the dose-response study, administration of 15 μg of Ex4 demonstrated a delayed onset and prolonged hypophagic effect in MFD and HFD rats (Fig. 2, A–E).

**Effect of High-Fat Diet on Liraglutide-Induced Hypophagia**

After 16 wk on their designated diet, MFD and HFD rats had greater body mass (F2,38 = 5.47; P < 0.05) and fat mass (F2,38 = 9.11; P < 0.05) than LFD rats, with no significant difference between MFD and HFD rats (Fig. 3A). Lean mass was similar among groups (Fig. 3A). Analysis of food intake during the 0–2- and 2–4-h intervals after liraglutide administration revealed no significant time × diet × drug interaction (F2,76 = 1.86; P > 0.05; Fig. 3, B and C). However, analysis of food intake during the 0–24-h, 24–48-h, 48–72-h, 72–96-h, and 96–120-h intervals after intraperitoneal administration of liraglutide did reveal a time × diet × drug interaction (F8,304 = 4.83; P < 0.05). During the 0–24-h and 24–48-h intervals, liraglutide-treated LFDRats, MFD, and HFD rats ate less than their saline-treated controls (P < 0.05; Fig. 3D). However, during the 48–72-h intervals, food intake was not significantly different between liraglutide- and saline-treated LFD rats, but was still significantly less in liraglutide-treated MFD and HFD rats compared with their saline-treated controls (P < 0.05), whereas the HFD rats were the only rats to maintain a significantly lower food intake compared with controls during the 72–96-h interval (Fig. 3D). During the 96–120-h interval, food intake was no longer significantly suppressed in LFD, MFD, or HFD rats (Fig. 3D). During the 24–48-h and 48–72-h intervals, liraglutide-treated HFD rats ate less than liraglutide-treated LFD rats (P < 0.05; Fig. 3D). Liraglutide treatment significantly lowered body mass 24 h following administration (diet × drug interaction; F2,76 = 3.46; P < 0.05), with all liraglutide-treated rats losing more mass than their saline-treated rats (P < 0.05), but no statistical difference was found between the liraglutide-treated groups (Fig. 3E). At the start of the liraglutide study, MFD and HFD rats had greater body mass and fat mass than LFD rats (Fig. 3A), suggesting that the obese state itself, as opposed to the chronic exposure to a high-fat diet, could mediate the duration of GLP-1r-mediated hypophagia. Therefore, we analyzed data from a subset of LFD, MFD, and HFD rats that had similar body (546 ± 20 g, 543 ± 21 g, and 536 ± 25 g, respectively, P > 0.05) and fat mass (99 ± 6 g, 106 ± 10 g, and 130 ± 16 g, respectively, F < 0.05). The reduction in food intake was significantly greater in the 10 and 33 μg/kg Ex4-treated HFD rats than MFD rats treated with a similar dose (P < 0.05; Fig. 1F).
respectively, $P > 0.05$; $n = 5$ per group) from the above-mentioned experiment. Additional analyses of food intake during the 48–72-h interval following liraglutide administration with these body mass- and fat mass-matched LFD, MFD, and HFD rats revealed significant anorectic effects in the liraglutide-treated MFD and HFD rats compared with saline-treated control rats (43% and 54% suppression of food intake, respectively), whereas this effect was absent in the LFD group (4% suppression of food intake; data not shown).

**Effect of High-Fat Diet on I3VT Ex4-Induced Hypophagia**

LFD and HFD rats were injected with saline or Ex4 (0.1 µg, I3VT), and food intake was monitored after 2, 4, and 24 h. Analysis of food intake during the 0–2- and 2–4-h intervals after Ex4 administration revealed no time × diet × drug interaction ($F_{1,20} = 0.98$, $P > 0.05$; Fig. 4, A and B). During the 0–24-h interval (main effect of drug, $F_{1,20} = 51.07$; $P <
Ex4-treated rats ate less than saline-treated controls independent of diet (P < 0.05; Fig. 4C).

**Effect of High-Fat Diet on Ex4 Pharmacokinetics**

Because Ex4 had a delayed but longer-lasting hypophagic effect in MFD and HFD rats compared with what occurred in LFD rats, we analyzed serum Ex4 levels 2, 4, and 24 h after administration of exogenous Ex4 in rats that had been on their designated diet for 11 wk. Serum Ex4 levels were significantly increased in all diets at 2 and 4 h following Ex4 administration (P < 0.05) and returned to baseline levels 24 h after intraperitoneal Ex4 injection (main effect of time; F3,168 = 52.87; P < 0.05; Fig. 5).

**Effect of High-Fat Diet on Glp1r and Gcg Expression**

Glp1r expression in the distal ileum and NTS of HFD rats was significantly higher and lower, respectively, compared with levels in LFD rats (P < 0.05; Fig. 6A). However, Glp1r expression in the hypothalamus and nodose ganglion did not differ significantly among groups (Fig. 6A). Ileal and NTS Gcg expression had an increased and decreased trend in HFD rats compared with LFD rats (P = 0.08 and P = 0.09, respectively;
DISCUSSION

The present data demonstrate that diets high in fat content change the temporal profile of hypophagia induced by exogenous long-acting GLP-1r agonists and that this is manifested by a delayed onset but longer-lasting anorexic action. In fact, the anorexic effect of a single administration of liraglutide was still present during the fourth day after administration in rats on a high-fat diet. The delayed onset occurs only following peripheral administration of GLP-1r agonists, and the longer-lasting hypophagia is not due to decreased clearance of the drugs and, thus, may relate to changes in tissue-specific GLP-1r function. These findings suggest that dietary composition impacts the anorectic actions of peripherally administered GLP-1r agonists.

Decreased breakdown of Ex4 or liraglutide and subsequent prolonged activation of GLP-1r could be a possible explanation for the longer-lasting anorexic actions of Ex4 or liraglutide observed in this study. Because Ex4 and liraglutide have greater stability in the circulation than GLP-1 (7), we hypothesized that physiological changes resulting from chronic consumption of a high-fat diet enhanced their stability even fur-
Fig. 4. High-fat diet does not alter time profile of I3VT Ex4-induced hypophagia. Caloric intake during the 0–2-h (A), 2–4-h (B), and 0–24-h (C) intervals following I3VT administration of either saline (S) or 0.1 μg Ex4 (Ex4). Rats were on their respective diet for 6 wk (n = 4–6/group). **P < 0.05, different letters indicate significant differences between treatment groups.

ther. However, serum Ex4 levels did not differ among LFD, MFD, and HFD rats, suggesting that differences in peptide clearance were not responsible for the observed differences in food intake suppression. Although there were no differences in serum Ex4 at 2 h postinjection, it is possible that up to that point, serum Ex4 may have been reduced, contributing to a delay in Ex4-induced hypophagia. However, Ex4 levels were not elevated at later time points. Thus, our data suggest that absorption, and subsequent delayed release of Ex4, by increased amounts of white adipose tissue in MFD and HFD rats is not involved in mediating the longer-lasting action of GLP-1r-mediated hypophagia during high-fat diet maintenance.

During the food intake studies, MFD and HFD rats had higher body mass than LFD rats, suggesting that an obese state itself could mediate the changed time course of GLP-1r-induced hypophagia. However, additional analyses of food intake during the third day following liraglutide administration using a subset of LFD, MFD, and HFD rats that were matched for body and fat mass suggest that the prolonged duration of hypophagia induced by exogenous long-acting GLP-1r agonists is independent of body and fat mass.

CNS GLP-1rs, most notably in the hypothalamic paraventricular nucleus (PVN) and the hindbrain, are implicated in the ability of GLP-1r agonists to decrease food intake (2, 7, 12, 15, 20, 24, 36, 39, 44, 50). However, pharmacological ablation of afferent C-type neural fibers using a capsaicin approach or a subdiaphragmatic vagal deafferentation approach partially blocks the ability of peripheral Ex4 to reduce food intake (27, 33, 46). This suggests that GLP-1r on sensory afferent neurons is at least important for the short-term anorectic function of GLP-1r agonists. In the present study, chronic HFD consumption decreased NTS but not nodose ganglion or hypothalamic *Glp1r* expression. In addition, central administration of Ex4 had similar anorectic potency in LFD and HFD rats. This indicates that during HFD-feeding, hypothalamic GLP-1r function appears normally responsive to Ex4 and suggests that functional changes are related but may not be limited to the level of the hindbrain. As the NTS is a relay for peripherally originating neuronal signals to be forwarded to higher brain centers, decreased NTS *Glp1r* expression might be associated with a reduced efficacy of Ex4 to lower food intake shortly after administration; however, this does not explain the longer-lasting effects. Thus, a speculative but plausible model is that the short-term effects of peripheral Ex4 or liraglutide are mediated through visceral afferent nerve signaling, while the longer-term effects result from movement of the agonists into the brain, where they can interact with central GLP-1r directly. Consistent with this idea, one study found that subdiaphragmatic vagotomy shifted the dose response curve of Ex4 and liraglutide to the right meaning that higher doses were necessary to get the same anorectic effects (27). Another study found that subdiaphragmatic vagotomy impairs early, but not later satiating effects of Ex4 (33). Further research is necessary to determine whether high-fat diets impair the ability of these long-acting agonists to directly activate vagal afferent neurons.

While these long-acting agonists can act via a GLP-1r-dependent sensory afferent pathway, they can rapidly cross the blood-brain barrier (BBB) (25, 29) and, therefore, have direct actions on the CNS that are independent of their actions on vagal afferents. Central resistance to the anorexigenic action of both leptin and insulin, both important regulators of long-term energy balance, has been hypothesized to be at least partially explained by reduced transport of leptin and insulin across the BBB during high-fat diet-induced and genetic obesity (10, 26). Thus, maintenance on a high-fat diet (and possibly the consequent obese state) might compromise the transport of Ex4 or liraglutide into the brain, although this was not directly tested here.

In rodents, activation of GLP-1r is accompanied by malaise and visceral illness, either assessed using a conditioned taste aversion paradigm or a pica response paradigm (i.e., the consumption of a nonnutritive substance) (28, 31, 47). Subdiaphragmatic vagotomy did not blunt the ability of peripheral Ex4 to induce nausea (28), indicating that the vagus nerve is not necessary for this ability. Thus, although not directly tested here, it is possible that high-fat diet maintenance induces...
stronger malaise side effects than low-fat diet maintenance, and future experiments are warranted to investigate this in more detail.

It has to be noted that, as indicated in Table 1, the fatty acid profile differs substantially between the high-fat diets used. In general, we observed similar efficacy of Ex4 and liraglutide to suppress food intake in MFD and HFD rats. However, during the 0–48-h interval, food intake was suppressed to a greater extent in 10 or 33 μg/kg Ex4-treated HFD rats than MFD rats treated with a similar dose (Fig. 1F). This suggests that different classes of fatty acids might have differential effects on GLP-1r-signaling during long-term high-fat diet consumption; however, this needs to be investigated in the future.

While it is easier to speculate as to why there was a delay in GLP-1r-agonist action, it is difficult to understand the longer-lasting effects. GLP-1r-mediated suppression of food intake and body mass, at least in the NTS, is dependent on an increase in cAMP-dependent PKA activity and an inhibition of AMPK activity (23). Leptin signaling is mediated, in part, by these same intracellular pathways, and consumption of a high-fat diet induces leptin resistance within days (40, 51). Furthermore, leptin and GLP-1 interact to suppress food intake (52). Although not directly tested here, it is likely that the MFD and HFD rats are leptin-resistant after 6 wk on the high-fat diet, which is when the rats were tested for the first time (51). Thus, the temporal profile of GLP-1r action during chronic high-fat diet consumption might be affected by both “initial” central leptin resistance and down-regulation of hindbrain Glp1r. However, as GLP-1rs are activated in higher brain centers (i.e., the PVN and reward-related brain areas) at a later stage, some of the central leptin resistance could potentially be overcome leading to a more prolonged suppression of food intake.

Knauf et al. (32) reported that 2-wk consumption of a high-fat diet increased brain stem Gcg mRNA levels in mice. Furthermore, our laboratory has observed a positive correlation between body weight and NTS Gcg expression after 4 wk on a high-fat diet (6). However, in the present study, NTS Gcg expression did not differ between LFD and HFD rats, nor did we observe a correlation between body mass and NTS Gcg expression within any group (data not shown). This might be explained by differences in diet exposure, as Gcg expression was analyzed after 20 wk of HFD consumption.

In conclusion, chronic consumption of a high-fat diet in rats delayed the onset but also prolonged the action of GLP-1r-mediated depression of food intake. The molecular mechanisms that lead to this effect remain to be determined. GLP-1r-based therapies are currently being used to treat T2D and overweight in humans (41). Although it remains to be seen whether our rodent data can be translated to the human situation, our data suggest that although diets high in fat content alter the temporal profile of GLP-1r-mediated hypophagia, in the end this alteration may potentially serve to benefit the weight-loss effects of the drug.

ACKNOWLEDGMENTS

We would like to thank members of the Seeley Laboratory for helpful discussions. M. Miller for help with animal handling, A. P. Chambers, H. Hoppert, and A. Lewis for help with tissue isolation, and E. Smith and B. Reedy for help with the Ex4 EIA and total GLP-1 analysis.

GRANTS

This work was supported by grants from National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (DK-017844 to S. C. Woods; DK-54080, DK-82480, DK-54890, and DK-056863 to R. J. Seeley; DK-082480 to D. A. Sandoval). J. D. Mul is supported by an American Society for Metabolic and Bariatric Surgery grant. D. P. Begg is supported by an National Health and Medical Research Council of Australia Early Career Fellowship (1013264).

DISCLOSURES

J. D. Mul is a paid speaker for Taconic. D. A. D’Alessio does consulting for Amylin, Lilly, Novo Nordisk and Takeda, research support from Ethicon Endosurgery and MannKind, and receives support for lectures from Merck. R. J. Seeley currently has stock/stock options with Zafgen and consults for Zafgen, Eli Lilly, and Ethicon Endo-Surgery. D. A. S’Alessio receives grant support from Ethicon Endo-Surgery and Novo Nordisk. The remaining authors disclose no conflicts.
AUTHOR CONTRIBUTIONS


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