Decreased gastric mechanodetection, but preserved gastric emptying, in CCK-1 receptor-deficient OLETF rats

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De Jonghe, Bart C., Andras Hajnal, and Mihai Covasa. Decreased gastric mechanodetection, but preserved gastric emptying, in CCK-1 receptor-deficient OLETF rats. Am J Physiol Gastrointest Liver Physiol 291: G640–G649, 2006. First published May 25, 2006; doi:10.1152/ajpgi.00109.2006.—Obese CCK-1 receptor-lacking Otsuka Long Evans Tokushima fatty (OLETF) rats are hyperphagic relative to control, nonmutant Long Evans Tokushima Otsuka (LETO) rats. This study sought to assess whether the overeating observed in OLETF rats is associated with changes in gastric emptying rates or detection of gastric volume. We performed experiments in both 12- and 29-wk-old OLETF and LETO rats to address possible alterations in gastric functions during the development of increased body weight and blood glucose abnormalities in OLETF rats. Gastric emptying of a 5-g solid chow test meal was not significantly different between strains at either 1, 2, or 4 h postmeal. When rats with ad libitum access to chow were tested, there were no significant differences in gastric emptying between strains at any time period despite OLETF rats consuming significantly more chow than LETO rats. Similar to solid food, 5-min gastric emptying of a 5-ml isosmotic and hyperosmotic saline or glucose load was not significantly different between strains. When the stomach was distended with a 15-ml semisolid chow load, there was no significance difference in emptying at either 1 or 2 h. No significant differences in gastric emptying were detected between 12- and 29-wk-old rats under any conditions. Both young and old OLETF rats, however, reduced sham intake significantly less compared with LETO rats during a brief period of gastric distension by 5- or 10-ml balloon inflation. Finally, OLETF rats showed decreased Fos expression in the nucleus of the solitary tract relative to LETO rats after an 8-ml gastric distension. These findings demonstrate that OLETF rats do not express deficits in controlling gastric emptying rates; however, they exhibit decreased behavioral and vagal responsiveness to gastric distension that may contribute to the increased meal size in these animals.

cholecystokinin; hyperphagia; cholecystokinin-1 receptor; c-Fos; gastric distension; sucrose; sham feeding

The OTSUKA LONG -EVANS TOKUSHIMA FATTY (OLETF) rat is an outbred mutant strain of the Long Evans Tokushima Otsuka (LETO) rat that lacks CCK-1 receptor expression entirely due to a spontaneous 6.8-kb mutation spanning the promoter and first two exon regions of the CCK-1 receptor gene (46). In addition to the use of these animals as a model of insulin resistance, due to their natural manifestation of hyperglycemia and non-insulin-dependent diabetes mellitus relative to age-matched LETO rats (16), OLETF rats are also currently under investigation as a model of obesity. OLETF rats exhibit an increased rate of weight gain relative to controls across their lifespan, with a marked elevation in body weight seen as early as 2–4 postnatal days (2, 27, 43). It is also known that OLETF rats are hyperphagic via increased meal size. This behavior has been attributed not only to deficits in CCK-related satiation mechanisms (27) but also to intestinal nutrient satiation signaling (5, 7, 43) and, more recently, to enhanced oral responsiveness to palatable stimuli (7, 13).

Gastric emptying is one mechanism through which CCK functions to reduce food intake (29). In nonmutant animals, CCK-1 receptor antagonists have been shown to attenuate the inhibition of gastric emptying by both exogenous CCK administration (12, 28) as well as gastric nutrient loads (26, 49). The mechanism for both of these effects appears to be largely mediated through CCK-1 receptor activation of vagal afferent signaling (38, 40). In addition, Schwartz et al. (39) observed enhanced and amplified vagal afferent activity due to the presence of a gastric load when CCK was simultaneously administered, implying that CCK and its receptors contribute to mecanoreception of gastric contents. In this regard, the CCK-1 receptor-deficient OLETF rat offers an ideal model to study gastric functions known to be mediated by CCK-1 receptors and at the same time may contribute to the understanding of the increased meal size in this strain.

Therefore, in the present study, we investigated whether, in addition to defects in intestinal nutrient and CCK satiation signaling deficits, OLETF rats also express impairments in CCK-mediated satiation via defective gastric contributions to meal termination. Accordingly, in the first series of experiments, we assessed gastric emptying rates of solid and liquid foods in OLETF and LETO rats using various feeding conditions and gastric load manipulations.

Diminished detection of gastric volume, due to a lack of gastric vagal afferent CCK-1 receptor activation (39), may also lead to the increased meal size in the OLETF rat. To examine this possibility, the second series of experiments examined the effects of stomach distension on sham feeding and gastric emptying. Finally, gastric distension has been shown to excite specific regions of the dorsal medulla controlling for meal size via vagal activation (39). Quantification of the immediate-early gene product Fos has been used as an indicator of neuronal activation stemming from vagal afferent transmission of such signaling (10, 21, 50). Thus, the final experiment examined whether or not OLETF and LETO rats differ in neuronal responsiveness to gastric distension by assessing Fos expression in select areas of the hindbrain. To control for the possible confounding gastroparetic effects resultant from non-insulin-
dependent diabetes mellitus development (17) in OLETF rats, experiments were performed in two age groups [12 wk (age 1) and 29 wk (age 2)], which represented nondiabetic and prediabetic OLETF animals unless otherwise indicated.

METHODS

Animals

Male OLETF and LETO rats were obtained as a generous gift of the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). Age 1 and age 2 rats (age 1: 423 ± 12.0 and 315 ± 6.0 g for OLETF and LETO rats, respectively; age 2: 557 ± 17.0 and 450 ± 6.4 g for OLETF and LETO rats, respectively) were used in these experiments. Separate groups of rats were used for each set of experiments within both age groups tested unless otherwise indicated. All animals were individually housed in mesh-floor, stainless steel hanging cages in a temperature-controlled vivarium while maintained on a constant 12:12-h light-dark cycle (lights on at 0600). Rats were handled daily for a minimum of 1 wk before the onset of experimental procedures. Tap water and pelleted rat chow (Purina 5001) were available ad libitum throughout the experiments. All protocols used were approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Procedures

Gastric fistula implantation. Rats designated for solid food emptying studies were fasted overnight and anesthetized before surgery via an intramuscular injection with 1.0 ml/kg of a mixture of ketamine HCl (100.0 mg/ml), xylazine (20.0 mg/ml), and acepromazine maleate (10.0 mg/ml) obtained from Burns Veterinary Supply (Rockville Centre, NY) and surgically implanted with chronic gastric fistulae according to Yox and Ritter (51). The inner flange of a gastric fistula (stainless steel, 13-mm length, 6-mm inner diameter and 8-mm outer diameter) was inserted through the ventral wall of the nonglandular portion of the stomach near the greater curvature and subsequently secured with a purse string suture. A piece of Marlex mesh was centered to adhere flush with the outer flange of the fistula. The nonflanged end of the fistula was then externalized through a left paramedian abdominal incision. A removable stainless steel screw inserted into the fistula blocked access to the stomach lumen between experiments. The peritoneum and abdominal muscles were simultaneously sealed with absorbable sutures postimplantation. The abdominal incision was closed using wound clips, which were removed 7 days postsurgery. Rats were allowed a minimum of 1 wk to recover from fistula implantation surgery before experimentation.

Gastric emptying of a 5-g solid chow meal in OLETF and LETO rats. Because of the known hyperphagic phenotype of the OLETF rat, this experiment controlled for increased meal size by limiting the size of the test meal to a known amount readily consumed by both OLETF and LETO rats within 1 h. Overnight (16 h)-fasted OLETF and LETO rats (n = 6–8 rats/strain at both age 1 and age 2) were presented with one preweighed 5-g pellet of rat chow for consumption. All rats had no food left in the cage before the assessment of emptying. Collected spillage was weighed to accurately measure how much of the 5-g meal was consumed. Gastric emptying of the chow consumed was measured at 1, 2, or 4 h postpresentation. Before the tests, the stainless steel screws occluding the gastric fistulae were removed, and the stomach contents were rinsed with warm tap water by continual flushing until the solution withdrawn was clear of ingested chow particles. Both recovered gastric contents and samples of pelleted chow were dried according to previously established procedures to isolate dry matter (DM) content (3). DM emptied was calculated by the following equation: DM emptied (%). 

Gastric emptying of solid chick in OLETF and LETO rats allowed ad libitum access to food. The rates of gastric emptying within a meal (i.e., when gastric contents accumulate as a result of ongoing ingestion) have been previously shown to greatly exceed the rate of nutrient emptying after meal termination (15, 48). Thus, in the hyperphagic OLETF rat, the greater magnitude of gastric fill due to increased meal size may result in increased emptying relative to LETO controls. To investigate this effect, we next allowed animals free access to chow, in contrast to the previous experiment, when they were fed with a restricted amount, for either 1, 2, or 4 h. After these periods, gastric emptying was measured. This design examined whether the increased food consumption by OLETF rats would increase the evacuation of gastric contents. Rats used in the previous experiment (n = 6–8 rats/strain at both age 1 and age 2) were also used in this study.

Gastric emptying of liquid solutions in OLETF and LETO rats. Because OLETF rats overconsume not only solid but also liquid foods, the following study examined gastric emptying of both isosmotic and hyperosmotic nutritive and non-nutritive liquid loads. To do so, we measured the gastric emptying of known liquid loads [isosmotic 5% saline (150 mmol/l), isosmotic 3.5% hyperosmotic 0.88 mmol/l), hyperosmotic 2.0% saline (347 mmol/l), and hyperosmotic 12.5% glucose (694 mmol/l)] in OLETF and LETO rats. Each of the four solutions was tested twice in all rats. During testing, 16-h overnight-fastad rats (n = 6–8 rats/strain at both age 1 and age 2) received a 5-ml volume of load containing 0.006% phenol red instilled into the rat’s stomach via oral gavage. After a 5-min emptying period, the remaining gastric contents were withdrawn, and the stomach was rinsed repeatedly with water until withdrawals were void of any visible phenol indicator. Gastric emptying was determined by dye-dilution spectrophotometry from absorption at 550 nm as previously described by our laboratory (14). Five-minute gastric emptying was determined from the following formula: liquid emptied (%) = [1 – (phenol red recovered from the stomach)/(phenol red in the instilled load)] × 100 (3).

Gastric emptying of a semisolid chow load in OLETF and LETO rats. For each of the prior solid chow gastric emptying experiments, rats freely ingested their gastric “load” before the measurement of emptying rates. On the basis of a previous report by Kaplan et al. (15) showing that the rate of oral delivery changes the rate of gastric emptying, we elected to test the rate of gastric emptying after a direct gastric infusion of a semisolid chow emulsion in the absence of postgastric feedback. A relatively large load of a semisolid chow mixture was chosen to examine the gastric emptying of a load with a high degree of gastric distension in addition to nutrient content. Specifically, a 15-ml load of semisolid [25% (wt/vol)] chow mixture homogenized in distilled water was directly instilled into the stomach through the gastric fistula. The remaining gastric contents were removed at 1 or 2 h after instillation of the gastric load, and emptying was determined using the DM method described above.

Sham feeding of sucrose in response to gastric distension in OLETF and LETO rats. This experiment assessed whether or not OLETF and LETO rats differ in their detection of gastric volume in the absence of postgastric feedback. To do this, we compared sham feeding of naïve OLETF and LETO rats (n = 8 rats/strain at age 1 and 6 rats/strain at age 2) in response to volumetric distension by an intraesophageal balloon. After a 16-h fast, the stainless steel screws occluding the gastric fistulae were removed, and the stomach contents were lavaged with warm tap water to ensure minimal gastric volume and distension upon the start of sham feeding. Rats were placed into Plexiglas sham feeding boxes and acclimated to the sham feeding procedure by presenting them with 0.3 M (10.2% sucrose for 90 min over several sessions until a stable baseline intake was reached (~3–4 sessions on consecutive days). Subsequently, on the tests, the effects of gastric distension on intake were evaluated. Different degrees of gastric distension were administered using an 8-Fr Foley catheter (Bardex, Bard, Covington, GA) with an inflatable tip. Before the presentation of sucrose, the inflatable end of the catheter was fed

GASTRIC EMPTYING AND DETECTION IN THE OLETF RAT

G641

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AJP-Gastrointest Liver Physiol • VOL 291 • OCTOBER 2006 • www.ajpgi.org
through a drainage tube attached to the gastric fistula and advanced 0.5–0.7 cm into the lumen of the stomach. The catheter was held in place by a rubber band attached to the external end, which prevented movement from the original insertion position. Five minutes later, rats were presented with burettes filled with 0.3 M sucrose solution. Ten minutes postpresentation of sucrose, the catheter was inflated (20-s inflation time) with either 5 or 10 ml of warmed 0.9% saline for a period of 20 min. After the catheter was deflated at 30 min (20-s deflation time), rats were allowed to sham feed sucrose for an additional 60 min to detect any compensatory changes in sham intake when the effects of distension were removed. Thus, rats had access to 0.3 M sucrose for a total of 90 min. Each distension load was given a minimum of 2 experimental days and was always bracketed by a nondistension experimental day when rats did not receive any load during sham feeding. These nondistension days served to assess possible baseline intake shifts due to distension on the previous experimental day. Sham intake was measured to the nearest 0.1 ml every 5 min. In all sham feeding tests, gastric drainage was collected in plastic graduated cylinders placed beneath the cages, and the volume was recorded at the experiment termination. In the event that the volume of fluid ingested was greater than the volume of gastric drainage, or if gastric drainage did not occur within 15 s of the start of sham feeding, data from that subject were discarded on the basis that the gastric fistula was not properly placed or functioning (51).

Analysis of c-Fos expression in the hindbrain of OLETF and LETO rats after gastric distension. A separate group of OLETF and LETO rats was used for the analysis of Fos expression in response to gastric distension. Overnight (16-h) food-deprived OLETF and LETO rats were removed from their home cages and placed in Plexiglass sham feeding boxes as described above in Sham feeding of sucrose in response to gastric distension in OLETF and LETO rats. Twenty minutes after the attachment of the drainage tube, an 8-Fr Foley catheter was inflated with warm tap water as in the previous experiment. Eight rats (n = 4 rats/strain) had their stomachs distended with 8 ml of warmed water for a period of 90 min, whereas six rats (n = 3 rats/strain) underwent all procedures as described above except that no inflation of the catheter occurred (sham distension). These methods have been previously described by van de Wall and colleagues with slight modifications (47).

Ninety minutes after the onset of gastric distension, all animals were deeply anesthetized and intracardially perfused using a 0.1 M phosphate buffer solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Whole brains were then removed, subsequently stored for 4 h in 4% paraformaldehyde, and finally transferred to 20% sucrose solution for overnight storage. Thirty-micrometer cryostat cut sections were processed for Fos-like immunohistochemistry (Fos-LI) as previously described (6). Stained brain sections were inspected microscopically, and counts of all Fos-LI nuclei were made. The counts were done manually by an individual blinded to the treatments. Fos-LI nuclei were counted bilaterally in the dorsal vagal complex (DVC), which comprised the nucleus of the solitary tract (NTS), area postrema (AP), area subpostrema (AsP), and dorsal motor nucleus of the vagus (DMV), at six levels of the dorsal hindbrain (−14.30, −14.08, −13.80, −13.68, −13.30, and −13.24 mm from bregma) corresponding to plate levels 76–71 according to the stereotaxic atlas of Paxinos and Watson (32). At minimum, three sections per brain level were analyzed for each rat. The presented data are the average numbers of Fos-LI cells within or across plate levels for each rat and treatment condition.

Determination of stomach weights. OLETF and LETO rats used in these experiments at age 2 (n = 16 rats/strain) were killed, and stomachs harvested after study completion. Briefly, the stomach was exposed via a midline celiotomy, ligated at the pylorus and cardia, resected, and weighed. The resected stomachs were then incised and scraped clean of any food particles. The empty stomachs were blotted to remove excess liquid and weighed (14).

Oral glucose tolerance test and insulin tolerance test. An oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed in a subset of rats (n = 7 rats/strain) within each age group after experimentation. For the OGTT, after a 16-h fast, an oral glucose load (2 g/kg) was delivered to each rat orally via latex gavage. For ITT, human regular insulin (0.75 U/kg body wt, Humalin R, Eli Lilly Japan, Kobe, Japan) was administered intraperitoneally to all rats. For both tests, blood glucose was measured before gavage or preinjection and at 30, 60, 90, and 120 min postglucose loading or insulin injection by a standard glucometer (LifeScan, One-Touch Basic). Animals were classified as diabetic if the peak level of plasma glucose was ≥300 mg/dl and the peak glucose level at 120 min was >200 mg/dl (16).

Statistical Analysis

For solid gastric emptying experiments, gastric emptying was analyzed using two-way repeated-measures ANOVA with strain and time as main factors. Food intake was analyzed using one- or two-way repeated-measures ANOVA where applicable. Liquid gastric emptying was examined using two-way repeated-measured ANOVA with strain and gastric load as the main factors. Gastric emptying of solid and semisolid chow is presented as the percentage of DM emptied from the stomach.

For sham feeding/gastric distension experiments, separate two-way repeated-measures ANOVAs were used to calculate the effects of distension treatments on individual 5-min intake bins in both OLETF and LETO rats using distension volume and time as the main effects. Quantification of Fos-LI nuclei was analyzed by two-way ANOVA with distension treatment (gastric distension vs. sham) and strain as main factors. Blood glucose in OLETF and LETO rats after the OGTT or ITT were compared using planned t-tests. For all experiments, ANOVA results were subsequently analyzed by Tukey’s honestly significant difference post hoc tests when appropriate. All data are expressed as means ± SE. Differences were considered statistically significant if P < 0.05. Statistical analyses were computed with PC-SAS (version 8.02, SAS Institute, Cary, NC).

RESULTS

Gastric Emptying of a 5-g Solid Chow Meal in OLETF and LETO Rats

Both OLETF and LETO within the two age groups consumed the entire 5-g chow meal presented, barring spillage. No strain differences in intake were noted at age 1 [4.4 ± 0.1 and 4.3 ± 0.1 g for OLETF and LETO rats, respectively, F(1,15) = 0.7, P = 0.274] or age 2 [4.7 ± 0.1 and 4.7 ± 0.1 g for OLETF and LETO rats, respectively, F(1,15) = 0.1, P = 0.988]. ANOVA results at age 1 showed no significant strain × time interaction [F(2,30) = 1.2, P = 0.251]. Gastric emptying increased across time [F(2,30) = 25.0, P < 0.001]; however, this was not significantly different between OLETF and LETO rats [F(1,15) = 0.8, P = 0.877] at either 1 h (40.2 ± 5.8% and 41.1 ± 4.1%, respectively), 2 h (55.2 ± 3.0% and 60.2 ± 2.2%, respectively), or 4 h (66.5 ± 2.1% and 68.3 ± 2.5%, respectively), postpresentation of a 5-g chow meal. Similarly, at age 2, no significant interaction effect for strain × time was observed [F(2,30) = 0.5, P = 0.783], whereas a significant main effect for time was noted [F(2,30) = 33.5, P < 0.001]. At age 2, gastric emptying was again not significantly different between OLETF and LETO rats [F(1,15) = 0.6, P = 0.854] at either 1 h (41.6 ± 3.2% and 41.8 ± 4.5%, respectively), 2 h (53.4 ± 2.3% and 50.5 ± 3.0%, respectively), or 4 h (68.7 ± 1.9% and 68.1 ± 1.8%, respectively) postpresentation of a 5-g chow meal.

AJP-Gastrointest Liver Physiol • VOL 291 • OCTOBER 2006 • www.ajpgi.org
OLETF rats consumed more food than LETO rats after an overnight fast. As expected, OLETF rats consumed significantly more chow than LETO rats at both age 1 (A, right) and age 2 (B, left); however, OLETF rats consumed significantly more chow than LETO rats at both age 1 (A, right) and age 2 (B, right). *P < 0.01 and ***P < 0.001, significantly different values between strains.

Figure 1B shows results from rats at age 2 after ad libitum access to chow for 1 h after an overnight fast. As expected, OLETF rats consumed more food than LETO rats [F(1,15) = 16.3, P < 0.01]. Gastric emptying of chow showed a main effect of time [F(2,30) = 51.7, P < 0.001] but not strain [F(1,15) = 0.2, P = 0.997]. No interaction between strain and time on gastric emptying was observed at age 2 [F(2,30) = 0.4, P = 0.644].

When rats were given a larger window of ad libitum access to chow at age 1, OLETF rats consumed significantly more chow than LETO rats [F(1,15) = 13.8, P < 0.001] across age 2 [F(2,30) = 33.9, P < 0.001]; however, no strain x time interaction was noted [F(2,30) = 0.6, P = 0.734]. Post hoc results showed increased chow intake in OLETF rats at the 1-h (P < 0.001), 2-h (P < 0.05), and 4-h (P < 0.01) access periods (Fig. 2A). At age 1, no main effect for strain [F(1,15) = 0.2, P = 0.812] on gastric emptying was observed (Fig. 2A), although a significant time effect was shown [F(2,30) = 20.1, P < 0.001]. No significant strain x time interaction [F(2,30) = 0.2, P < 0.838] was evident for ad libitum chow gastric emptying at age 1.

At age 2, ANOVA results again showed significant main effects for both strain [F(1,15) = 26.9, P < 0.001] and time [F(2,30) = 31.8, P < 0.001] on chow intake after ad libitum access periods, although there was no strain x time interaction [F(2,30) = 0.2, P = 0.799]. Post hoc results showed that OLETF rats consumed more chow than control LETO rats at the 1-, 2-, and 4-h (all P < 0.01) access periods (Fig. 2B). Figure 2B also illustrates that no significant main effect of strain [F(1,15) = 0.2, P = 0.824] was observed for gastric emptying at age 2; however, a main effect for time on gastric emptying was shown [F(2,30) = 18.3, P < 0.001].

Gastric Emptying of Liquid Loads in OLETF and LETO Rats

No main effects of strain [age 1: F(1,15) = 0.1, P = 0.996; age 2: F(1,15) = 0.2, P = 0.945] or gastric load [age 1: F(1,15) = 0.4, P = 0.639; age 2: F(1,15) = 0.4, P = 0.688] were noted in 5 ml emptying of liquid loads among either age group tested. Specifically, gastric emptying of a 5-ml load of isosmotic saline (age 1: 76.2 ± 2.0% and 72.2 ± 3.7% for OLETF and LETO rats, respectively; age 2: 59.1 ± 2.0% and 62.5 ± 2.8% for OLETF and LETO rats, respectively), hyperosmotic saline (age 1: 59.3 ± 3.9% and 62.2 ± 4.6% for OLETF and LETO rats, respectively), and hypotonic saline (age 1: 59.5 ± 3.6% and 56.3 ± 2.8% for OLETF and LETO rats, respectively) was not significantly different between strains.
respectively; age 2: 50.7 ± 3.3% and 52.7 ± 4.1% for OLETF and LETO rats, respectively), or hyperosmotic glucose (age 1: 48.2 ± 5.3% and 44.5 ± 3.3% for OLETF and LETO rats, respectively; age 2: 44.2 ± 2.7% and 42.4 ± 4.6% for OLETF and LETO rats, respectively) were no different between strains.

Gastric Emptying of Semisolid Chow in OLETF and LETO Rats

No significant main effect of strain \([F(1,10)=1.1, P=0.179]\) in gastric emptying of a 15-ml load of a 25% chow mixture load was noted at age 2 between OLETF and LETO rats at either 1 h (35.7 ± 4.2% and 32.6 ± 2.4% for OLETF and LETO rats, respectively) or 2 h (52.9 ± 3.4% and 46.1 ± 3.1% for OLETF and LETO rats, respectively).

Sham Feeding of Sucrose in Response to Gastric Distension in OLETF and LETO Rats

Figure 3 depicts the 20-min gastric distension effects on sham intake of 0.3 M sucrose within 5-min bins over a 90-min sham feeding session in OLETF or LETO rats at age 1 or age 2. Figure 3A shows the results of gastric distension in OLETF rats at age 1. Two-way ANOVA results showed significant main effects for gastric distension \([F(2,21)=12.3, P<0.001]\) and time \([F(17,357)=8.8, P<0.01]\) as well as a significant distension \(\times\) time interaction \([F(34,357)=3.54, P<0.01]\). Post hoc analyses of these results showed the effects on sham intake to be confined to time periods where distension occurred. Specifically, no response to 5-ml distension was noted in OLETF rats at age 1, whereas significant suppressions in sham intake relative to baseline were noted during 10-ml distension conditions at the 15-, 20-, and 25-min time points \((P<0.01\) for all 3 time points). Figure 3B shows age 1 results in LETO rats receiving a 20-min gastric distension. Two-way ANOVA in LETO rats at age 1 revealed significant main effects for both gastric distension \([F(2,21)=24.1, P<0.001]\) and time \([F(17,357)=14.3, P<0.001]\) and also a significant distension \(\times\) time interaction \([F(34,357)=7.7, P<0.001]\). Post hoc analyses of these results (depicted in Fig. 3B) showed significant reductions in sham intake only within the 20-min distension period. However, unlike in OLETF rats, LETO rats showed a significant suppression in intake in response to both 5- and 10-ml distension volumes. In particular, intake reductions were noted at the 15-min \((P<0.05\) and \(P<0.001\) for 5- and 10-ml distensions, respectively), 20-min \((P<0.01\) and \(P<0.001\) for 5- and 10-ml distensions, respectively), and 25-min \((P<0.05\) and \(P<0.01\) for 5- and 10-ml distensions, respectively) time points.

Gastric distension effects on sham intake in OLETF rats at age 2 are shown in Fig. 3C. Similar to the results at age 1, OLETF rats at age 2 showed significant main effects of distension \([F(2,15)=15.7, P<0.001]\) and time \([F(17,255)=14.3, P<0.001]\) and, in addition, a significant distension \(\times\) time interaction \([F(34,255)=2.6, P<0.01]\). Intake at the 15-min time point was significantly reduced in both 5-ml \((P<0.05)\) and 10-ml \((P<0.01)\) distension conditions, whereas intake at 20 min was significantly reduced \((P<0.01)\) in the 10-ml distension condition only.

Figure 3D illustrates the effects of gastric distension on sham intake in LETO rats at age 2. LETO rats at age 2 showed significant main effects of distension \([F(2,15)=26.8, P<0.001]\) and time \([F(17,255)=45.2, P<0.001]\) and a significant distension \(\times\) time interaction \([F(34,255)=11.9, P<0.001]\). Unlike in OLETF rats, post hoc analyses in LETO rats showed intake reductions during distension at three time points during
distention: 15 min (P < 0.05 and P < 0.001 for 5- and 10-ml distensions, respectively), 20 min (P < 0.01 and P < 0.01 for 5- and 10-ml distensions, respectively), and 25 min (P < 0.05 and P < 0.01 for 5- and 10-ml distensions, respectively).

Analysis of c-Fos Protein Expression in the Hindbrain of OLETF and LETO Rats Due to Gastric Distension

Analyses of c-Fos expression in the hindbrain of OLETF and LETO rats revealed significant differences within the NTS region of the DVC. Specifically, a strain main effect was noted for both sham [F(1,4) = 22.1, P < 0.01] and 8-ml distension [F(1,6) = 6.94, P < 0.05] conditions, indicating a decreased average of NTS Fos-LI in OLETF rats relative to LETO rats (Table 1). Post hoc analyses within NTS plate levels showed significant differences between OLETF and LETO rats. OLETF rats distended with an 8-ml gastric balloon showed a significant decrease in NTS Fos-LI at 13.24-mm (P < 0.01) and 13.68-mm (P < 0.01) levels compared with LETO controls. Under nondistended sham conditions, OLETF rats showed a decreased Fos expression at 13.24-mm (P < 0.05) and 13.68-mm (P < 0.05) levels relative to LETO rats.

There were no significant differences in Fos expression between OLETF and LETO rats within either treatment group at the 14.30-mm (P = 0.986), 14.08-mm (P = 0.955), or 13.80-mm (P = 0.810) plate levels. Additionally, we did not observe any significant differences in Fos expression in any other area of the DVC including the DMV, AP, or AsP between OLETF and LETO rats at any plate level examined (all P > 0.05).

OGTT and ITT

As shown in Table 2, at both age 1 and age 2, OLETF rats showed increased blood glucose levels relative to LETO rats after glucose challenge. At age 1, significant increases were noted in OLETF rats at 30 and 60 min (P < 0.001 for both time points) compared with LETO rats, with the highest blood glucose peak at 30 min (173 ± 5.1 vs. 110 ± 5.5 mg/dl in OLETF and LETO rats, respectively). At age 2, all time points measured postglucose challenge were significantly higher in OLETF rats (P < 0.01 for all time points), with the highest blood glucose peaks occurring at 60 min (253 ± 30.0 vs. 111 ± 5.9 mg/dl in OLETF and LETO rats, respectively).

After an acute insulin injection at age 1, OLETF rats showed an attenuated decrease in blood glucose 120 min postinsulin injection compared with LETO animals (70 ± 3.5 vs. 51 ± 1.5 mg/dl in OLETF and LETO rats, respectively, P < 0.05; Table 2). At age 2, OLETF rats showed significantly higher blood glucose at 90 min postinsulin injection compared with LETO rats.
GASTRIC EMPTYING AND DETECTION IN THE OLETF RAT

Table 1. Counts of Fos-LI nuclei within the NTS of 8-ml gastric-distended or sham-distended OLETF and LETO rats

<table>
<thead>
<tr>
<th>Distance From Bregma, mm</th>
<th>Distension</th>
<th>Sham Distension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OLETF</td>
<td>LETO</td>
</tr>
<tr>
<td>−14.30</td>
<td>42.7±4.5</td>
<td>44.1±2.7</td>
</tr>
<tr>
<td>−14.08</td>
<td>108.2±14.3</td>
<td>101.9±5.9</td>
</tr>
<tr>
<td>−13.80</td>
<td>119.5±8.5</td>
<td>132.4±16.5</td>
</tr>
<tr>
<td>−13.68</td>
<td>121.2±22.9</td>
<td>216.2±17.5†</td>
</tr>
<tr>
<td>−13.30</td>
<td>156.0±28.4</td>
<td>162.3±14.2</td>
</tr>
<tr>
<td>−13.24</td>
<td>57.7±6.2</td>
<td>102.5±15.7†</td>
</tr>
<tr>
<td>Average</td>
<td>129.7±15.9</td>
<td>164.4±8.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE of numbers of Fos-like-immunoreactive (Fos-LI) nuclei in the hindbrain of Otsuka Long Evans Tokushima fatty (OLETF) and Long Evans Tokushima Otsuka (LETO) rats. OLETF rats showed decreased average nuclei of the solitary tract (NTS) Fos-LI nuclei for both sham and 8-ml distension conditions relative to LETO rats. Post hoc analyses within NTS plate levels showed that OLETF rats distended with an 8-ml gastric balloon showed decreased NTS Fos expression at −13.24- and −13.68-mm levels compared with LETO controls, whereas in nondistended sham animals, OLETF rats showed decreased Fos expression at −13.30- and −13.68-mm levels. *P < 0.05 and †P < 0.01, significantly different values between OLETF and LETO rats under distension or sham distension conditions.

Table 2. Blood glucose levels after OGTT or ITT in OLETF and LETO rats at age 1 and age 2

<table>
<thead>
<tr>
<th>Age 1</th>
<th>OLETF</th>
<th>LETO</th>
<th>Age 2</th>
<th>OLETF</th>
<th>LETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>86.7±2.2</td>
<td>88.2±2.9</td>
<td>81.5±5.5</td>
<td>82.2±1.5</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>173.0±5.1</td>
<td>110.0±5.5</td>
<td>194.3±20.6</td>
<td>110.2±5.8‡</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>161.3±4.8</td>
<td>105.6±2.4</td>
<td>253.2±30.0</td>
<td>111.9±5.9‡</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>127.3±15.5</td>
<td>99.0±2.1</td>
<td>207.3±20.3</td>
<td>101.8±4.8‡</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>92.8±2.3</td>
<td>88.8±2.0</td>
<td>140.0±11.5</td>
<td>91.8±3.8‡</td>
<td></td>
</tr>
<tr>
<td>ITT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>84.4±3.2</td>
<td>79.7±2.4</td>
<td>83.3±2.3</td>
<td>77.3±1.3</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>72.3±3.0</td>
<td>72.3±2.0</td>
<td>69.4±4.4</td>
<td>59.5±6.4</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>63.5±3.5</td>
<td>63.4±6.0</td>
<td>59.5±5.0</td>
<td>48.8±4.4</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>67.6±4.3</td>
<td>57.7±1.7</td>
<td>53.4±4.9</td>
<td>35.0±1.8†</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>70.0±5.2</td>
<td>51.0±1.5*</td>
<td>46.3±4.5</td>
<td>39.7±3.7</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of blood glucose levels (in mg/dl). An oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed within each age group [12 wk (age 1) and 29 wk (age 2)] after experimentation. For OGTT, a glucose load (2 g/kg) was orally delivered after rats were fasted for 16 h. For ITT, all rats received an intraperitoneal injection of insulin. For both tests, blood glucose was measured before glucose loading or preinjection and at 30, 60, 90, and 120 min postloading or postinjection. *P < 0.05, †P < 0.01, and ‡P < 0.001, significant strain differences in blood glucose levels within each age group tested.

Our gastric emptying data appear to contrast with the expected outcome of accelerated gastric emptying in a CCK-1 receptor-deficient animal. An increased rate of emptying would be predicted according to the known role of exogenous CCK to inhibit gastric emptying of solid and liquid nutrients (8, 14, 29, 41) via CCK-1 receptors as well as from studies observing heightened gastric emptying of liquid nutrients by acute CCK-1 receptor blockade in normal rats (26, 35).

Data from other laboratories have shown decreased duodenal lipid-induced gastric acid secretion (44) and increased susceptibility to gastric mucosal lesions in the OLETF rat (31). More relevant to the present work, however, is that OLETF rats displayed delayed gastric emptying of a methylcellulose load compared with LETO rats (45). In that report, the authors showed no differential strain reduction in gastric emptying due to an acute administration of corticotropin-releasing factor and the muscarinic receptor antagonist atropine. A closer inspection of the magnitude of these effects, however, shows a much larger, and almost complete, abolishment of gastric emptying in the OLETF rat from atropine (45). It is not clear from the analysis reported whether this increased degree of suppression was significantly higher in OLETF rats. Such a scenario would be suggestive of decreased parasympathetic control of gastric emptying in the OLETF rat and may explain these observations.

Ohta et al. (30) extended these findings by showing that OLETF rats exhibit delayed gastric emptying in response to a caloric liquid gastric load. It was also theorized that sympathetic nervous function may be enhanced in OLETF animals compared with LETO animals, as indicated by a decreased responsiveness to reserpine-induced gastric emptying acceleration (30). Nonetheless, no studies to date have directly examined the possibility of altered gastric parasympathetic or sympathetic innervation in the OLETF rat.

In the present study, gastric emptying of both caloric and noncaloric, as well as hyperosmotic and isosmotic, gastric

rats (53 ± 4.1 vs. 35 ± 2.1 mg/dl in OLETF and LETO rats, respectively, P < 0.01) as well as a nonsignificant trend of an overall attenuation of decreased blood glucose due to insulin relative to LETO rats.

**Determination of Stomach Weight**

After OGTT and ITT administration at age 2, animals were killed and stomachs were resected. OLETF rat stomachs were significantly heavier than LETO stomachs [F(1,30) = 35.4, P < 0.01, 2.6 ± 0.1 vs. 2.0 ± 0.1 g for OLETF and LETO rats, respectively]. However, when values were corrected for body weight (body weight: 665.1 ± 16.0 vs. 547 ± 11.5 g for OLETF and LETO rats), the relative stomach sizes of OLETF and LETO rats did not differ (stomach weight: 0.39 ± 0.02 vs. 0.37 ± 0.02 g/100 g body wt for OLETF and LETO rats, respectively, P = 0.968).

**DISCUSSION**

The present findings indicate that OLETF rats do not show deficits in gastric emptying of either solid or liquid loads relative to LETO rats regardless of age and the progression of blood glucose impairments. Specifically, when OLETF rats consumed either a chow meal of equal or greater size within the same allotted meal period, gastric emptying was equal to that of LETO controls. Similarly, applying gastric distension via intragastric instillation of a relatively high volume load did not produce differential gastric emptying between strains. OLETF and LETO rats were also shown to have equal gastric emptying of both isosmotic and hyperosmotic nutritive and non-nutritive gastric loads across both age groups tested. In contrast, when the volumetric effects of gastric mechanodetection were isolated by inflation of an intragastric balloon, we observed reduced feeding responses within OLETF rats relative to LETO in both young and older animals. Furthermore, after gastric distension alone, OLETF rats showed marked decreases in Fos expression compared with LETO rats in select regions of the hindbrain known to facilitate the vaga response to changes in gastric volume.

AJP-Gastrointest Liver Physiol • VOL 291 • OCTOBER 2006 • www.ajpgi.org
loads were no different between OLETF and LETO rats, indicating that fluid emptying in the OLETF rat is also intact. It is worth noting that gastric emptying of a non-nutrient liquid load in CCK-2 receptor knockout mice is enhanced (24), although the precise mechanism behind this phenomenon is not known. Furthermore, gastric CCK-2 receptor mRNA in OLETF rats has been shown to be upregulated relative to LETO rats (23). Therefore, the possibility that putative deficits in gastric emptying in the OLETF rat due to the lack of CCK-1 receptor are compensated for by enhanced CCK-2 receptor activation cannot be discounted.

When a large liquid chow mixture was directly instilled into the stomach, we did not detect any differences in gastric emptying between OLETF and LETO rats. It has previously been reported that OLETF rats tend to have higher wet weights of stomachs at 12–14 wk of age (30), which parallels their increased body weight. We extended these findings by observing slightly increased dry stomach weights in OLETF rats at ~35 wk of age (age of death after age 2); however, when values were corrected for body weights of the animals at this age, this difference became negligible. Nonetheless, despite this apparent increased raw gastric size, our relatively large 15-ml load was not able to produce any distinguishable difference in gastric emptying between OLETF and LETO rats, suggesting that OLETF rats are able to maintain normal gastric emptying rates of a caloric load even when given a volume of distension likely close to the maximum gastric capacity under ad libitum feeding.

Additionally, Schwartz et al. (43) have shown that liquid nutritive gastric preloads are largely equal in their ability to suppress subsequent food intake, whereas duodenal preloads have distinctively diminished satiating effects in OLETF rats compared with LETO rats. Our data compliment these results by revealing that gastric emptying of a nutrient load in OLETF and LETO rats showed no apparent differences, regardless of the amount of chow consumed, present in the stomach, or administered in the liquid or solid phase. Gastric emptying of a nutrient load is distinguished by a period of an increased emptying rate during periods of gastric fill, such as within a meal or resultant from gastric nutrient infusion, whereas emptying subsequent to meal termination is maintained at a slower, constant tempo (15). While this work does not address potential deficits in initial rates of emptying, our results show clearly that gastric emptying in OLETF rats after meal termination is unchanged relative to LETO rats.

Isolating the volumetric component of distension via intragastric balloon inflation allowed us to examine the feeding responsiveness to a fixed volume of gastric distension in OLETF and LETO rats. Our results showed clear differences in intake patterns within OLETF and LETO rats during periods of gastric distension. Specifically, OLETF rats showed no response to 5-ml distension at age 1 and reduced intake at only one time point during distension at age 2, whereas LETO rats reduced intake from baseline at multiple time points during 5-ml distension at both ages. When distended with a 10-ml gastric volume, responses were similar in OLETF and LETO rats at age 1; however, the magnitude of intake attenuation in response to distension appeared to be greater in LETO rats relative to OLETF rats. Likewise, at age 2, the effects of 10-ml distension persisted for a longer period in LETO rats than in OLETF rats. These results suggest that OLETF rats have decreased sensitivity to gastric volumes during feeding and thus may require a relatively greater degree of volumetric distension to reduce food intake compared with LETO rats. This observation parallels our recent finding showing that OLETF rats exhibited a diminished responsiveness to intestinal nutrient infusion in a similar sham feeding design (7). In this context, prior reports have identified the vagal signal induced by stomach distension to be largely a function of mechanospecific receptors (20), in contrast to duodenal vagal afferents, which respond to both mechanos- and chemosensation (42). In general, of the two main classes of vagal afferent endings [intranganglionic laminar endings (IGLEs) and intramuscular arrays (IMAs)], IMAs have been shown to primarily mediate signals of stretch and length change within the stomach, whereas IGLEs function more in response to more direct muscular contraction (33). Of particular relevance to our findings is the recent report (9) showing that knockout animals lacking the neurotrophin (NT)-4 gene exhibit deficient intestinal IGLE innervation, which results in short-term satiation deficits. In contrast, NT-4 knockout mutants have been shown to be hypersensitive to CCK-induced satiation mediated through CCK-1 receptor activation (4). Thus, it is possible that diminished IGLE responsiveness due to a congenital lack of the CCK-1 receptor in the OLETF rat may contribute to the increased food intake in these animals. Given the present sham feeding design, however, it is unlikely that duodenal IGLEs play a significant role because no intestinal nutrient feedback was elicited. Nonetheless, when considering the functional differentiations in gastric and duodenal vagal innervation in an intact animal, one possible explanation for our sham feeding results is that gastric vagal mechanosensitivity is diminished in the OLETF rat, which would explain the attenuated decrease in intake due to stomach distension. Alternatively, a simpler explanation may be that the same degree of balloon inflation may not translate to the same degree of distension detection, due to the increased stomach size in the OLETF rat.

However, our gastric emptying results would not support the latter explanation because both small and large amounts of gastric nutrient volumes did not produce variable gastric emptying in any of our experiments at either age tested.

The suppressive effects of gastric distension on food intake have been shown to be largely mediated by activation of both gastric and hepatic branches of the vagus nerve (for a review, see Ref. 34). Our final experiment addressed whether the diminished feeding response to gastric distension in OLETF rats could be explained, at least partially, through decreased vagal activation of distension signals. Gastric distension induces Fos within the NTS region of the DVC (19, 47), an area established as the primary termination site for vagal afferent input from the stomach (50). Furthermore, distension-induced Fos expression in the NTS is abolished via vagotomy (21, 25). The present results of decreased DVC Fos expression within the NTS suggest that OLETF rats exhibit diminished vagal signaling induced by gastric distension relative to control LETO rats. This is in agreement with previous data from our laboratory (6) as well as others (11) showing decreased Fos expression in the enteric plexus, nodose ganglia, and hindbrain of OLETF rats compared with LETO rats. In addition, levels of Fos neurons in LETO rats are comparable to Fos counts observed by other laboratories using a similar degree of gastric distension (37).
Although CCK-1 receptors have been shown to participate in vagal responses after stomach distention, the precise degree of this participation has not been clear. Recently, van De Wall et al. (47) reported that lorglumide did not diminish the expression of Fos induced by 2-ml distention; however, it completely reversed the enhancement of distension-induced Fos expression by CCK. This suggests that lorglumide specifically reduces the response of vagal afferents to distension. The contributions by specific vagal afferent fiber populations in mediating gastric distension effects may vary according to the distension volume. It is possible that the relatively large 8-ml volume of gastric distension used in our study may result in the involvement of CCK-1 receptor activity not captured when smaller distension volumes were tested. Alternatively, the effect may be independent of CCK-1 receptors and due to a direct effect of reduced vagal transmission of distension signals. A detailed analysis of multiple levels of gastric distension in the OLETF rat would be necessary to test this hypothesis. It is also worth mentioning that our Fos results do not indicate whether the observed decreased neuronal response is a contributor or an artifact of the spontaneously increased meal size in the OLETF rat. An analysis of gastric distention-induced Fos expression using OLETF rats previously pair fed to LETO controls to limit meal size may be useful in answering this question.

Recent work by Reidelberger et al. (36) has provided interesting data focusing on peripheral versus central feeding effects of blood-brain barrier-permeable and non-permeable CCK-1 receptor antagonists. From these data, it appears likely that blockade of central or vagal CCK-1 receptors may be involved in separate, cooperative processes that lead to an overall increase in food intake in nonmutant animals. Indeed, the present Fos results would support peripherally associated CCK-1 receptor deficits in distension signaling. However, there is also a report (21) showing that cells expressing gastric distension-induced Fos in the NTS activate projections that extend beyond the hindbrain, to forebrain structures such as the paraventricular and supraoptic nuclei of the hypothalamus. In this context, alterations in neuropeptide Y signaling within the dorsomedial and arcuate nucleus of the hypothalamus have been previously implicated in hyperphagia in the OLETF rat (1). Thus, it is possible that beyond the vagal deficits described here, select hypothalamic nuclei implicated in gastric distension controls may be an additional contributor to the aberrant feeding behavior in the OLETF rat.

It may also be argued that our findings of slightly decreased NTS Fos expression in sham-treated OLETF rats compared with LETO rats may be indicative of a decreased responsiveness in general and not limited to gastric volume stimuli. This explanation is unlikely for two reasons. First, the actual raw total NTS Fos counts for the sham treatment were between 10 and 20 fold less than those in distension treatments. Although statistically different between strains, the small number of Fos-positive nuclei is rather indicative of a slightly lower background in OLETF rats. This may be attributed to a lower activity of OLETF rats during testing because these animals have been shown to be hypoactive (18). Second, the noted differential strain effects under gastric distension are of such exceedingly high magnitude relative to sham conditions that any slight baseline differences of a few Fos counts under sham conditions have little impact on the significance of distension effects between strains. This suggests that we did indeed observe specific differential effects of NTS Fos expression attributed to the gastric distension treatment and not a generalized lower degree of Fos expression in OLETF rats compared with LETO rats.

To summarize, our findings reveal that OLETF rats, despite showing increased food intake, do not express deficits in their ability to control gastric emptying across multiple levels of gastric capacitance. Nevertheless, OLETF rats do show diminished feeding responses and neuronal activation induced by gastric distension. Thus, it is unlikely that hyperphagia in these animals involves deficient gastric emptying; however, decreased gastric mechanosensation and detection of gastric volume, in combination with previously described satiation defects, may facilitate overconsumption. The present findings seem to be discordant with work using acute CCK-1 receptor antagonism and may suggest gastric and neuronal alterations in the OLETF rat not directly specific to CCK-1 receptor deficiency as possible mechanisms.

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GRANTS

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