A high-fat diet regulates gastrin and acid secretion through primary cilia

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ABSTRACT **The role of primary cilia in the gastrointestinal tract has not been examined. Here we report the presence of primary cilia on gastric endocrine cells producing gastrin, ghrelin, and somatostatin (Sst), hormones regulated by food intake. During eating, cilia in the gastric antrum decreased, whereas gastric acid and circulating gastrin increased. Mice fed high-fat chow showed a delayed decrease in antral cilia, increased plasma gastrin, and gastric acidity. Mice fed high-fat chow for 3 wk showed lower cilia numbers and acid but higher gastrin levels than mice fed a standard diet, suggesting that fat affects gastric physiology. *Ex vivo* experiments showed that cilia in the corpus responded to acid and distension, whereas cilia in the antrum responded to food. To analyze the role of gastric cilia, we conditionally deleted the intraflagellar transport protein Ift88 (*Ift88*$^{-/fl}$). In fed *Ift88*$^{-/fl}$ mice, gastrin levels were higher, and gastric acidity was lower. Moreover, gastrin and Sst gene expression did not change in response to food as in controls. At 8 mo, *Ift88*$^{-/fl}$ mice developed foveolar hyperplasia, hypergastrinemia, and hypochlorhydria associated with endocrine dysfunction. Our results show that components of food (fat) are sensed by antral cilia on endocrine cells, which modulates gastrin secretion and gastric acidity.—Saqui-Salces, M., Dowdle, W. E., Reiter, J. F., Merchant, J. L. A high-fat diet regulates gastrin and acid secretion through primary cilia. *FASEB J.* 26, 3127–3139 (2012). www.fasebj.org**

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The gastric epithelium is composed of two functional and histologically distinct compartments: the proximal corpus (oxyntic) mucosa characterized by the presence of acid-producing parietal cells and the distal endocrine mucosa (antrum) where secretion of the hormone gastrin occurs (**Fig. 1*A***). Low gastric pH functions as an antimicrobial barrier (1), and chronic changes in gastric acidity are known to alter gut microbiota (2). Furthermore, loss of acidity due to parietal cell atrophy or dysfunction is an initial step in the development of gastric neoplasia (3, 4). In addition to acid and digestive enzyme production, the stomach secretes approximately 20 different peptides and hormones. The function of most is still undefined.

The best characterized of the gastric hormones is gastrin produced by antral G cells (5). Gastrin stimulates parietal cells in the corpus to secrete acid in response to food intake. It is also considered a growth factor because it stimulates gastric cell proliferation and differentiation and has been implicated in cancer progression (6, 7). In addition to its role in the stomach, gastrin stimulates the endocrine and secretory pancreas (8, 9), the gallbladder (10), and the heart (11). Gastrin expression and secretion are inhibited by somatostatin (Sst; refs. 12–14), produced in the stomach by D cells found in the antrum and corpus (15, 16). In addition to gastrin, Sst also regulates ghrelin (17). Ghrelin is secreted by the endocrine X cells (also called A-like and P/D1 cells) of the stomach, and it modulates food intake (18, 19), the accumulation of fat mass (20), glucose homeostasis (21), and the immune system (22) and CNS (23). Its expression follows a circadian pattern (24) and circulating ghrelin peaks just before food intake (25, 26).

Cilia are organelles that cells use to sense their immediate environment (27, 28). They are composed of a microtubule axoneme built on the basal body, the main component of which is the mother centriole present during interphase. Most tissues are capable of making cilia, although the percentage of ciliated cells varies greatly with tissue type. Formation of the ciliary axoneme requires intraflagellar transport (Ift) proteins, such as intraflagellar transport protein 88 (Ift88), and transporter proteins, such as kinesin-II and its

Abbreviations: ac-Tub, acetylated α-tubulin; GI, gastrointestinal; Ift, intraflagellar transport; Ift88, intraflagellar transport protein 88; Shh, Sonic hedgehog; Sst, somatostatin; TFF2, trefoil-factor 2; UEA1, *Ulex europaeus* agglutinin 1

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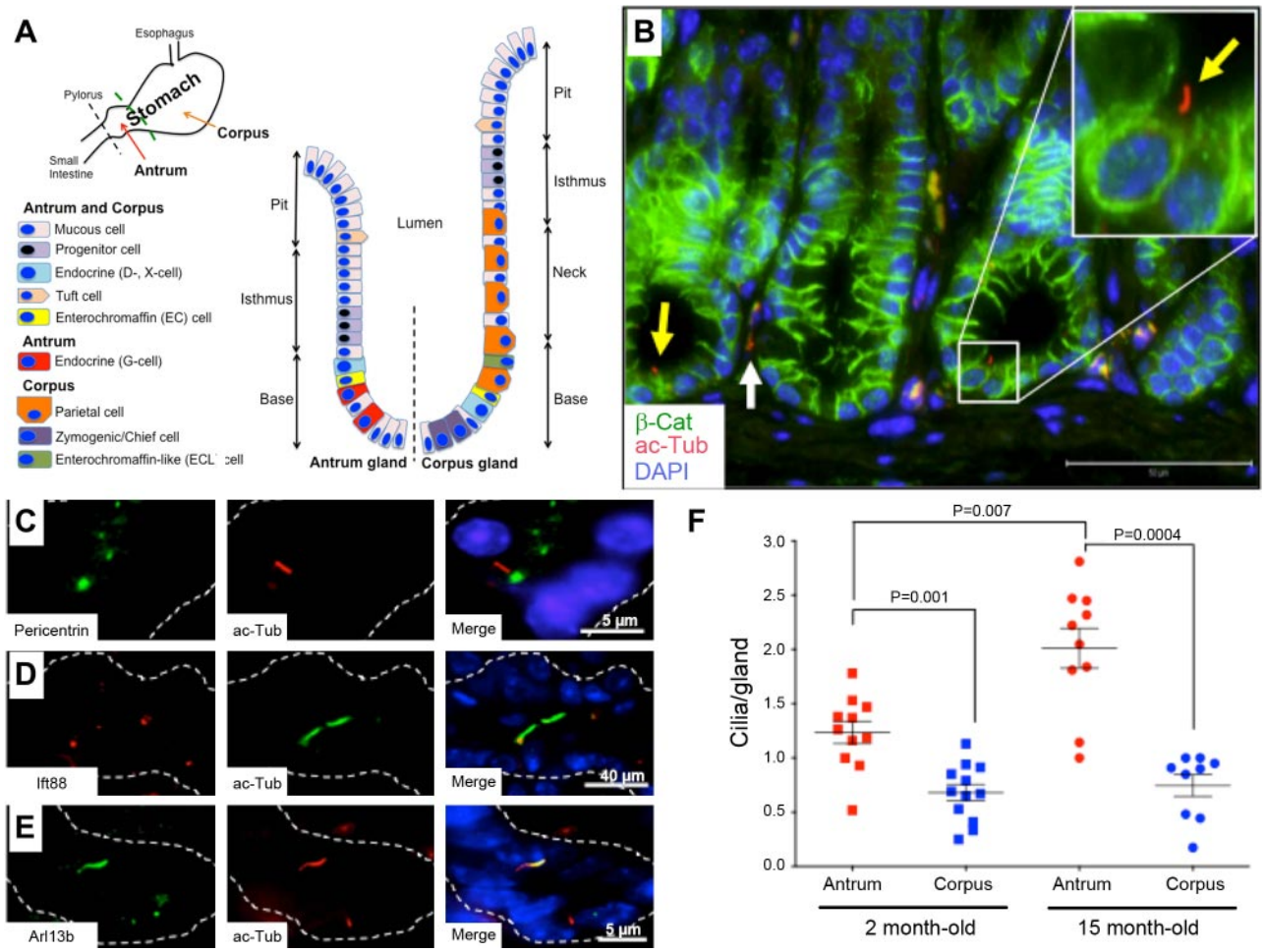

Figure 1. Epithelial cells in the stomach exhibit primary cilia. *A*) Representation of the gastric antral and corpus glandular structure. *B*) ac-Tub (red) staining in the antral gastric mucosa identified cilia in mesenchymal (white arrow) and epithelial cells (yellow arrows and inset, labeled with β-catenin, green). Nuclei were labeled with DAPI (blue). *C–E*) To confirm the ciliary structure, centrosome marker pericentrin (*C*, green), intraflagellar transport protein Ift88 (*D*, red), and the ciliary membrane marker Arl13b (*E*, green) were costained with ac-Tub. Nuclei were labeled with DAPI (blue). White dashed lines indicate epithelium basal membrane. *F*) Ciliated cells were counted in the gastric antra (red) and corpi (blue) of 2-mo-old (squares) and 15-mo-old (circles) mice. $n = 6$ females and 6 males for the 2-mo-old group; $n = 6$ females and 4 males for the 15-mo-old group.

subunit (Kif3a) (28). Select components that mediate signaling pathways such as those for hedgehog and platelet-derived growth factor receptor α, localize to cilia. In addition, certain hormone receptors, such as the Sst receptor 3 (29, 30) and leptin receptor (31), also localize to cilia. Some of these cilia-associated signaling pathways have previously been shown to operate in the gastrointestinal (GI) tract. However, the presence of cilia in the luminal GI had not been studied. Here we demonstrate the presence of primary cilia in the gastric epithelium and investigate their role in mediating gastric regulation by food intake.

MATERIALS AND METHODS

Animals and tissue samples

All animal handling and procedures were performed according to the University of Michigan Committee on Use and Care of Animals and the Institutional Animal Care and Use Committee at the University of California, San Francisco. The stomachs and intestines from age- and gender-matched C57BL/6J (wild type), *Gast*$^{-/-}$, *Sst*$^{-/-}$, and conditional null mice for *Kif3a* and *Ift88* were analyzed. The sonic hedgehog (*Shh*) promoter was used to drive the *Cre* coding sequence (32). Conditional deletion was achieved by crossing *Kif3a*$^{tm2.1Gsn/+}$ (*Kif3a*$^{/+}$);*Shh*$^{tm1(EGFP/cre)Cj}$ (*Shh-Cre*); *Gt(ROSA)26Sor*^{tm1Sor} (*R26R*) or *Ift88*$^{tm1.1Bky/+}$ (*Ift88*$^{-/+}$); *Shh-Cre;R26R* to *Kif3a*$^{tm2Gsn/tm2Gsn}$ (*Kif3a*$^{lox/lox}$) (33) or *Ift88*$^{tm1Bky/tm1Bky}$ (*Ift88*$^{lox/lox}$) (34) mice. *Kif3a*$^{-/lox}$;*Shh-Cre* (*Kif3a*$^{-/fl}$) and *Ift88*$^{-/lox}$;*Shh-Cre* (*Ift88*$^{-/fl}$) were compared with their *Cre*-negative littermates (control).

Stomachs were opened along the greater curvature, and one half was separated into antrum and corpus for RNA or protein extraction. The other half was processed for histological analysis by rinsing in PBS, fixing in buffered 4% formaldehyde solution overnight at 4°C, and embedding in paraffin or fixing for 10 min in 4% paraformaldehyde, incubating in 20% sucrose overnight at 4°C, and embedding in O.C.T. compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA).

For refeeding experiments, equivalent numbers of age-matched male and female mice were housed at no more than

2 mice/cage and prevented from feeding in cages without bedding with free access to water. Food pellets were removed at the start of the dark cycle. After 16 h of food withdrawal, free access to food was allowed (time 0). Mice were fed with either standard chow (Lab Diet 5L0D; PMI Nutrition International, Inc., St. Louis, MO, USA) or high-fat chow (D12492; Research Diets Inc., New Brunswick, NJ, USA). Mice were euthanized, and their stomachs were resected and weighed. The amount of food ingested was determined by normalizing the stomach weight to total body weight.

Gastric acid determination

Stomachs were resected and opened along the greater curvature. The gastric content was collected in 2 ml of 0.9% NaCl (pH 7.0) to determine the acid concentration by titration with 0.005 N NaOH (35) and normalized to total body weight (μEq of H^+/g).

Blood glucose levels

Mice fed with high-fat chow and control mice fed with standard chow for 3 wk were weighed weekly. Glucose levels were determined after 5 h of food withdrawal by tail blood sampling using Accu-chek Aviva test-strips and reader (Roche Diagnostics, Indianapolis, IN, USA).

ELISA for plasma gastrin

Blood was collected in cold heparin-coated tubes, and the plasma fraction was prepared by centrifugation for 5 min at 2000 *g*. The samples were kept at −80°C until analysis. The ELISA kit for human gastrin (catalog no. ADI-900−026; Enzo Life Sciences, Farmingdale, NY, USA) was used following the manufacturer's instructions, after the sample was diluted 1:4 in assay buffer.

***Ex vivo* model**

We used a modification of the protocol described by Dixit *et al.* (36). In brief, age- and gender-matched mice were prevented from feeding as described above, stomachs were collected, and any remaining gastric content was removed by flushing with cold PBS. The duodenal end was ligated, and the stomachs were filled *via* the esophagus until fully distended. The liquid remained in the stomach without esophageal ligation. This was defined as full capacity (average 850 μl). Half the volume was achieved immediately by removing half the volume. Stomachs were filled with aerated Krebs' solution consisting of 118 mM NaCl, 4.7 mM KCl, 1.0 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 11.0 mM glucose, and 2.5 mM $CaCl_2$ at pH 7.4 (control); Krebs' solution supplemented with additional 0.2 M glucose; and Krebs' solution acidified to pH 5.0 and pH 3.0 with acetic acid. For stomachs filled with standard and high-fat chow, chow pellets were homogenized with water until the mixture could be aspirated with a 16-gauge syringe. Full and half-filled stomachs were then immersed in an aerated Krebs' buffer bath at 37°C for 30 min. After incubation, the stomachs were opened and processed as described above.

Immunohistochemistry

Gastric sections were blocked for 30 min with 20% donkey serum in TBS (pH 7.4) plus 0.1% Triton X-100 (TBS-T). The excess blocking solution was removed, and primary antibodies were applied for 2 h at room temperature or 4°C overnight incubation. The samples were washed with TBS-T 3 times for 5 min each and then incubated with the corresponding secondary antibodies.

Antibodies used in this study were the following: mouse anti-α-acetylated-tubulin (1:500; Sigma-Aldrich, St. Louis, MO), rabbit anti-serotonin (1:200; Sigma-Aldrich), rabbit anti-pericentrin (1:150; Abcam, Cambridge, MA, USA), rabbit anti-chromogranin A (1:100; Abcam), rabbit anti-mucin5A (1:50; Abcam), rabbit anti-mucin 6 (1:20; Abcam), rabbit anti-β-catenin (1:200; Abcam), goat anti-ghrelin (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-gastrin (1:500; DakoCytomation, Glostrup, Denmark), Alexa 488 conjugated-*Griffonia simplicifolia* lectin II (1:1000; Vector Laboratories, Olean, NY, USA), *Ulex europaeus* agglutinin 1 (UEA1; 1:200, Vector Laboratories), rabbit anti-trefoil-factor 2 (TFF2; 1:2000; a gift from Andrew S. Giraud, Royal Children's Hospital, Parkville, VIC, Australia; ref. 37), mouse anti-H^+/K^+-ATPase α-subunit (1:1000; Medical and Biological Laboratories, Nagoya, Japan), rabbit anti-Ift88 (1:250; a gift from Bradley K. Yoder, University of Alabama at Birmingham, Birmingham, AL, USA; ref. 38), rabbit anti-intrinsic factor (1:200; a gift from David H. Alpers, Washington University, St. Louis, MO, USA; ref. 39), and rabbit anti-Arl13B (1:1000; a gift from Tamara Caspary, Emory University, Atlanta, GA, USA; ref. 40). Appropriate conjugated secondary antibodies (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) were used for fluorescence. Acetylated α-tubulin (ac-Tub) antibody was conjugated to DyLight-594 using the DyLight Microscale Antibody Labeling Kit (Thermo Scientific, Waltham, MA, USA). Sections were counterstained with nuclear DAPI. For light microscopy staining, the mouse-on-mouse MM HRP-Polymer Kit (Biocare Medical, Concord, CA, USA) was used. Microscopic analysis was performed using an Olympus BX60 fluorescent microscope or Olympus Fluoview 500 confocal microscope (Olympus, Tokyo, Japan).

Western blot

Stomachs were collected, cut along the greater curvature, and washed in cold PBS, and corpi and antra were separated and snap-frozen in liquid nitrogen. Samples were stored at −80°C until analyzed. Proteins were prepared by boiling for 15 min in 0.2 M acetic acid, followed by tissue homogenization and centrifugation for 14 min at 14,000 *g*, as described previously (41). The supernatant was collected, and proteins were resolved on 18% Tricine gels, transferred to PVDF membranes, and incubated with somatostatin-28 (1:2000; Abcam) or somatostatin (1:500; Millipore, Temecula, CA, USA). After transfer, the gels were stained for 10 min with Coomassie Blue. Proteins were visualized using the SuperSignal West Pico chemiluminescent substrate (Thermo Scientific, Rockford, IL, USA) after incubation with peroxidase-conjugated goat anti-rabbit antibody (1:2000; Santa Cruz Biotechnology). Films were scanned, and the resulting images were analyzed using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). Gels from 3 independent sets of mice were analyzed, and band density was expressed as relative units normalized to a 30-kDa protein band on the Coomassie Blue gel considered as the loading control.

Quantitative RT-PCR

Gastric mucosal samples were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) followed by phenol-chloroform RNA extraction and purification with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). First-strand cDNA was synthesized using iScript (Bio-Rad Laboratories, Hercules, CA, USA) accord-

ing to the manufacturer's protocol, and triplicates for each sample were amplified by quantitative PCR in a Bio-Rad iCycler using SYBR Green. The primer sequences for PCR amplification were as follows: *gastrin*, forward 5′-ACACAACAGCCAACTATTC-3′ and reverse 5′-CAAAGTCCATCCATCCGTAG-3′; *Sst*, forward 5′-TCTCGATCGTCCTGGCTTTGG-3′ and reverse 5′-TCATTCTCTGTCTGGTTGGGCTC-3′; *ghrelin*, forward 5′-GAGGACAAGCAGAAGAGACAGAGG-3′ and reverse 5′-GGCTGAGGCGGATGTGAGTTC-3′; *H^+/K^+-ATPase α-subunit*, forward 5′-TGTACACATGAGAGTCCCCTTG-3′ and reverse 5′-GAGTCTTCTCGTTTTCCACACC-3′; *H^+/K^+-ATPase β-subunit*, forward 5′-AACAGAATTGTCAAGTTCCTC-3′ and reverse 5′-AGACTGAAGGTGCCATTG-3′; *HPRT*, forward 5′-AGGACCTCTCGAAGTGTTGGATAC-3′ and reverse 5′-AACTTGCGCTCATCTTAGGCTTTG-3′; and *Ift88*, forward 5′-AGTGATATCCGGTACAAATGATGGAAAATGTTCA-3′ and reverse 5′-GAGTGATTTCCTCAGTATCATAGGCTGGGTTGTA-3′.

Statistics

Numbers of cilia were evaluated by counting cilia axonemes in ≥45 well-oriented glands in the antrum and corpus of each stomach. Gastrin (G)-, ghrelin (X)-, and Sst (D)-positive cells were counted at ×600 view on ≥30 well-oriented glands/sample. Gene expression was normalized to HPRT, and changes were calculated using the $2^{-\Delta\Delta CT}$ method (42). Data are presented as means ± SE or SD as indicated and were analyzed by 1-way ANOVA, Mann-Whitney, or Student's *t* tests using Prism (GraphPad Software, Inc., La Jolla, CA, USA) software.

RESULTS

To determine whether cilia are present in the epithelium of the mouse GI tract, we used antibodies to proteins of the ciliary axoneme to identify these organelles within the stomach, small intestine, and colon. Ac-Tub-labeled axonemes were present in both the gastric epithelium and mesenchyme (Fig. 1*B*). Because ac-Tub recognizes other intracellular structures containing stabilized microtubules, we used multiple markers to identify the ciliary structure by costaining ac-Tub with the centrosome marker pericentrin (ref. 43 and Fig. 1*C*), Ift88 (ref. 38 and Fig. 1*D*), and the cilia membrane marker ADP-ribosylation factor-like 13b (Arl13b; ref. 40 and Fig. 1*E*). Gastric ciliated cells were present on both the antral and oxyntic mucosa, and no gender differences were identified. Cilia were more abundant in the antrum, and their numbers increased with age (Fig. 1*F*). Although numerous ciliated cells were observed in the intestinal mesenchyme, none were identified in the mucosa of the small intestine (Supplemental Fig. S1*A*) or colon (Supplemental Fig. S1*B*). In rodents, the forestomach, lined with squamous epithelium, also exhibited primary cilia on cells comprising the basal layer (Supplemental Fig. S1*C*).

To identify which gastric cell types possess cilia, we costained for ac-Tub and known gastric epithelial markers (**Table 1** and Fig. 1*A*). Ac-Tub did not label one cell type exclusively, indicating that cilia in the gastric epithelium might have different functions, depending on the cell type. In addition to the solitary primary cilia on scattered epithelial cells, ac-Tub was also present in chemosensory tuft cells (Table 1, Fig. 1*A*, and ref. 44). Most of the ciliated cells were positive for the endocrine cell marker chromogranin A (Supplemental Fig. S1*D*). In the corpus, >75% of the cells producing the peptide ghrelin (X cells; Supplemental Fig. S1*E*) or Sst (D cells; Supplemental Fig. S1*F*) exhibited primary cilia. In the antrum, >80% of the gastrin-secreting cells (G cells; Supplemental Fig. S1*G*) and antral X and D cells also exhibited cilia.

To determine whether hormone levels regulate the appearance of cilia of cells in the stomach, we analyzed the presence of cilia in gastrin-deficient (*$Gast^{-/-}$*) and *$Sst^{-/-}$* mice. As a result of the loss of Sst, *$Sst^{-/-}$* mice are hypergastrinemic (15). We did not find differences in cilia numbers in the antrum or corpus of *$Gast^{-/-}$* or *$Sst^{-/-}$* mice compared with their littermate controls (Supplemental Fig. S2), suggesting that neither the

TABLE 1. *Gastric epithelial cells presenting cilia*

Cell type	Marker	Location
>70% presenting cilia		
G cells	Gastrin	Antrum
X cells (A-like cells)	Ghrelin	Corpus/antrum
D cells	Somatostatin	Corpus/antrum
<30% presenting cilia		
Mucous neck	GSII, Muc 6	Corpus
Zymogenic/chief	Pepsinogen/intrinsic factor	Corpus
Negative for cilia		
Enterochromaffin-like cell	Histamine	Corpus
Enterochromaffin cell	Serotonin	Corpus/antrum
Parietal[a]	H^+,K^+-ATPase	Corpus
Tuft[a]	TRPM5, GNAT3, DCLK1	Corpus/antrum
Mucous pit	Mucin 5AC	Corpus/antrum

GSII, *Griffonia simplicifolia* lectin II; Muc, Mucin; TRPM5, transient receptor potential cation channel subfamily M member 5; GNAT3, guanine nucleotide-binding protein, α-transducing 3 (α-gustducin); DCLK1, doublecortin-like kinase 1 (DCAMKL1). aThese cells stain for ac-Tub but are not ciliated.

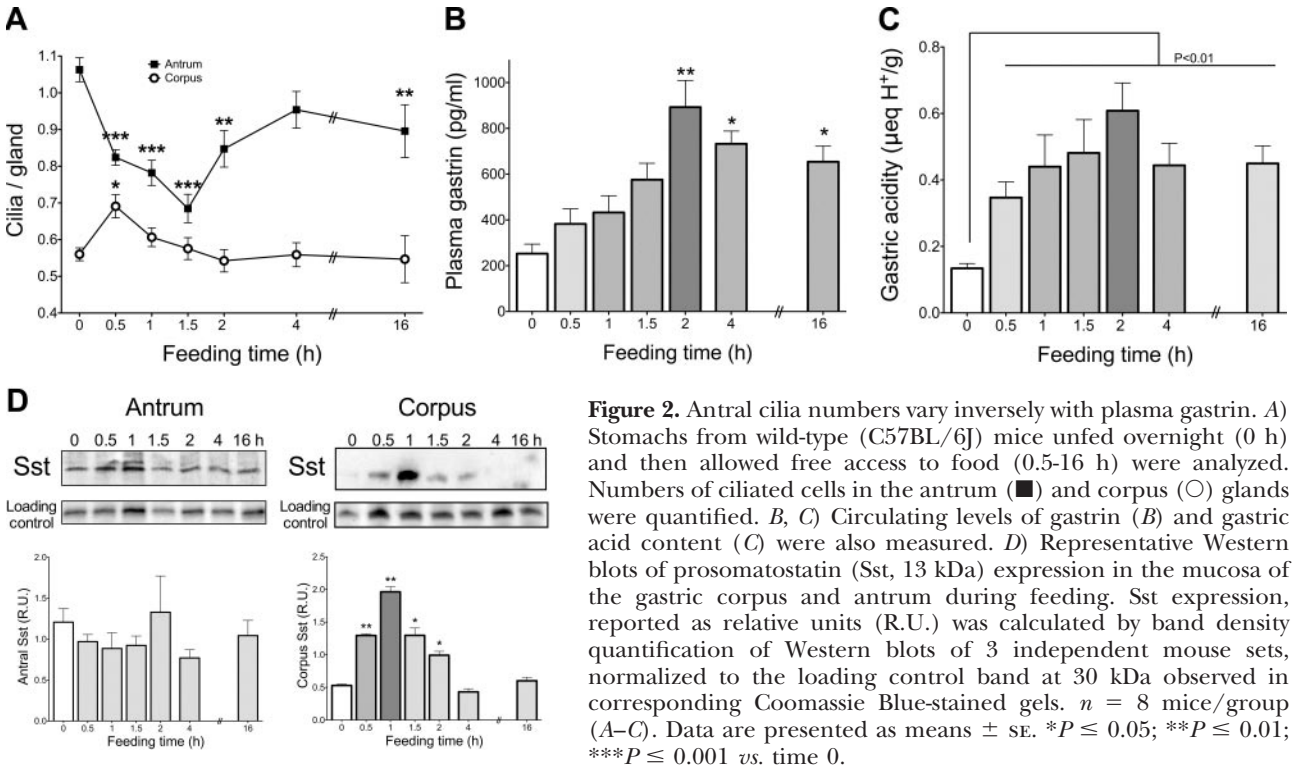

Figure 2. Antral cilia numbers vary inversely with plasma gastrin. *A*) Stomachs from wild-type (C57BL/6J) mice unfed overnight (0 h) and then allowed free access to food (0.5-16 h) were analyzed. Numbers of ciliated cells in the antrum (■) and corpus (○) glands were quantified. *B*, *C*) Circulating levels of gastrin (*B*) and gastric acid content (*C*) were also measured. *D*) Representative Western blots of prosomatostatin (Sst, 13 kDa) expression in the mucosa of the gastric corpus and antrum during feeding. Sst expression, reported as relative units (R.U.) was calculated by band density quantification of Western blots of 3 independent mouse sets, normalized to the loading control band at 30 kDa observed in corresponding Coomassie Blue-stained gels. $n = 8$ mice/group (*A–C*). Data are presented as means ± SE. $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$ *vs.* time 0.

absence of Sst and gastrin nor higher gastrin levels regulate the number of cilia in the stomach.

Because gastric endocrine cell function is tightly linked to food intake, nutrients, and acid secretion (45, 46), we hypothesized that primary cilia on these enteroendocrine cells respond to luminal signals, *e.g.*, food intake. Therefore, we tested whether the numbers of gastric cilia changed with food withdrawal and feeding. Basal levels were defined as those of mice that were unfed for 16 h (labeled as feeding time 0 h). Mice fed for 16 h showed similar levels for all the parameters analyzed compared with mice that were fed without food withdrawal (data not shown). Enumeration of the number of primary cilia demonstrated a transient increase in corpus cilia 0.5 h after refeeding (**Fig. 2*A***, open circle). In contrast, the number of ciliated cells in the gastric antrum decreased significantly by 1.5 h and began to recover by 2 h (Fig. 2*A*, solid square). However, the number of ciliated antral cells in fed mice did not recover to the levels in unfed mice after 16 h of feeding. To determine whether the number of cilia varied in response to endocrine cell numbers, we quantified the number of immune-positive cells for gastrin, ghrelin, and Sst. The numbers of gastric G, X, and D cells detected did not change when unfed mice were compared with fed mice (**Table 2**). Even when there was a reduction in the proportion of all ciliated antral endocrine cells, the G cells accounted for the most significant change in cilia numbers, from 83 to 64% in unfed and fed mice, respectively (Table 2). Although the GI epithelium is highly proliferative, endocrine cells do not undergo mitosis, and their turnover in the stomach is ~2 mo (47). Thus, our observation that changes in the number of ciliated cells, particularly on G cells, suggested that endocrine cells in the stomach are capable of dynamic cilia presentation in response to food intake.

As expected, with food intake, we observed a steady increase in plasma gastrin, which peaked at 2 h (Fig. 2*B*) concomitantly with a peak in gastric acidity (Fig. 2*C*), whereas antral cilia numbers reached their nadir by 1.5 h (Fig. 2*A*). Paracrine release of Sst is known to inhibit gastrin and acid secretion. Therefore, we analyzed the gastric mucosa for the Sst propeptide, which migrates at ~13 kDa on Western blots. It is assumed that in the antrum, open D cells (48) regulate nearby G

TABLE 2. *Numbers of endocrine cells in mice unfed or fed for 16 h*

Cell type	Location	Unfed (cells/gland)	Fed (cells/gland)	*P*, Mann-Whitney
G cells	Antrum	1.487 ± 0.23	1.346 ± 0.23	0.2786
X cells	Antrum	0.752 ± 0.11	0.799 ± 0.15	0.7209
	Corpus	1.028 ± 0.13	1.087 ± 0.36	0.5054
D cells	Antrum	0.215 ± 0.11	0.236 ± 0.05	0.5104
	Corpus	0.113 ± 0.09	0.091 ± 0.09	0.7984

Values are means ± SD; $n = 7$/group.

cells. However, we found no significant change in the levels of Sst during the time period of dramatic cilia reduction and gastrin increase (Fig. 2*D*, left panel). In the corpus, Sst peptide peaked at 1 h, which followed the transient increase in cilia numbers in the corpus (Fig. 2*D*, right panel). Therefore, circulating gastrin and acid secretion peaked after the decrease in antral cilia number and reduced Sst peptide levels in the corpus but not in the antrum. These results further supported the observation in hormone-deficient mice that cilia numbers were not regulated by endocrine secretion and suggested that the presence of food in the stomach regulated cilia numbers.

To test whether food composition regulates cilia, we analyzed the gastric response of mice fed with the high-fat chow and compared it with the response to the standard chow. The stomachs were weighed to quantify the amount of food ingested and normalized to body mass. With standard chow, the amount of ingested food peaked at 1 h. The stomach weights remained constant between 1 and 2 h and then decreased after 2 h, indicating gastric emptying (**Fig. 3*A***, open triangles). When mice were fed high-fat chow, the maximum amount of food ingested peaked by 2 h and did not decrease significantly over the entire 16-h experimental period (Fig. 3*A*, solid circles). The number of cilia in the corpus of the high-fat chow group increased for 1 h (Fig. 3*B*, open circles), whereas in mice fed standard chow, the increase occurred for only 0.5 h. In the antrum, the number of cilia varied slightly by 2 h and decreased only after 4 h (Fig. 3*B*, solid squares). This pattern was different from the changes observed with standard chow (Fig. 2*A*) for which a steady decrease in cilia number was observed for 1.5 h. With standard chow, plasma gastrin levels steadily increased during the first 2 h and remained constant after 4 h (Fig. 2*B*). When mice were fed high-fat chow, gastrin levels increased 2-fold during the first hour, dropped to basal at 1.5 and 2 h, and then increased after 4 h (Fig. 3*C*), the same time at which the antral cilia number started decreasing. Gastric acidity increased with high-fat chow intake until 4 h, but, in contrast with intake of standard chow, acidity remained elevated at 16 h (Fig. 3*D*).

We found interesting the fact that feeding high-fat chow resulted in a delayed decrease in cilia numbers, along with altered gastrin and acid secretion. To further analyze whether fat ingestion had a permanent effect on gastric physiology and whether our observations were not a result of hyperphagia induced by the novelty of the food, we fed mice with high-fat chow for 3 wk. After 3 wk of consuming high-fat chow, males (n=3) increased their weight by 22%, whereas females

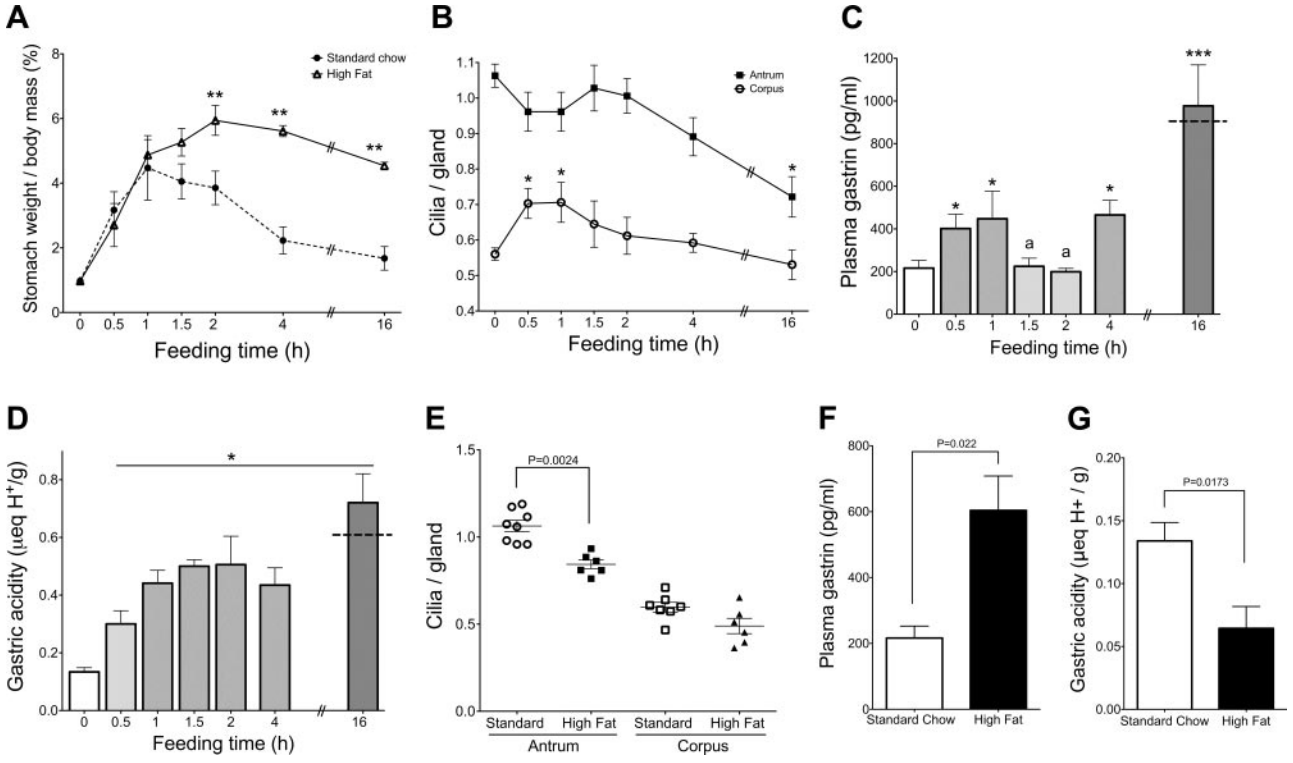

Figure 3. A high-fat diet retards decreases in cilia, gastrin, and acid secretion. *A*) Food intake, assessed as stomach weight at the time of analysis normalized to body weight of mice fed with standard chow (○) or high-fat chow (△) after overnight food withdrawal (0 h). *B*) Quantification of antral (■) and corpus (○) cilia in mice fed high-fat chow. *C*, *D*) Plasma gastrin (*C*) and gastric acidity (*D*) were determined in mice fed high-fat chow. Dashed lines indicate maximum gastrin (*C*) and acidity (*D*) achieved with standard chow (see Fig. 1*B, C*). *E–G*) Mice fed high-fat chow for 3 wk showed lower numbers of antral cilia (*E*), higher levels of circulating gastrin (*F*), and lower acid secretion (*G*) than mice fed standard chow. Mice were age- and gender-matched. n = 8 for mice fed standard chow; n = 6 for mice fed a high-fat diet. For the 3-wk feeding experiment, n = 8 for the standard chow group; n = 6 for the high-fat group. Data are presented as means ± SE. $^*P \leq 0.05$, $^{**}P \leq 0.01$ *vs.* time 0; $^aP \leq 0.05$ *vs.* same time point for standard chow.

(n=3) gained 10% weight. The basal number (after overnight food withdrawal) of ciliated cells in the antrum of mice fed high-fat chow was significantly lower than that of mice fed standard chow (Fig. 3*E*). The corpus cilia also diminished, but this change was not statistically significance (P=0.0734). Gastrin levels were higher in mice fed high-fat chow (Fig. 3*F*), but acid levels were lower (Fig. 3*G*). Blood glucose levels did not change significantly in mice fed high-fat chow for 3 wk (124±10 mg/dl for males and 115±6 mg/dl for females to 144±20 and 137±17 mg/dl, respectively). These results confirmed that consumption of a high-fat diet altered gastrin and acid secretion in the stomach.

Gastrin expression is regulated by diverse signals including nutrients, hormones, neurotransmitters, pH, and stomach distension (46). To evaluate whether cilia in the stomach were responsive to distension, we used an *ex vivo* model in which excised stomachs were filled to their full or half capacity with Krebs' solution (control). To test whether the cilia responded to the presence of glucose or pH, the glucose content or pH of the Krebs' solution was modulated. Using the *ex vivo* model, we observed elevated numbers of ciliated cells in both antrum and corpus compared with the cilia numbers *in vivo*. When stomachs were filled to full capacity for 30 min, the numbers of cilia in the corpus increased (**Fig. 4*A***), similar to what we observed in the corpus when the mice were fed for 0.5 h after food withdrawal. The presence of glucose had no effect on cilia presentation apart from what was observed with full distension (Fig. 4*A*). When the pH of the solution was decreased from 7.4 to 5.0 or 3.0, the expected increase in corpus cilia numbers was not observed with full distension (Fig. 4*A*). These results suggested that presentation of cilia in the corpus depends on distension and gastric acidity. Interestingly, none of the three conditions evaluated affected the number of antral cilia (Fig. 4*B*).

We then queried whether the antral cilia changes observed in mice when feeding were the result of luminal sensing of specific food components or the result of signals intrinsic to the body, in which case we expected not to observe changes in antral cilia in the *ex vivo* model. When we filled stomachs to full capacity with either standard chow or high-fat chow, we observed a significant decrease in the cilia number in the corpus (Fig. 4*C*). Standard chow has a pH of 5.5 and an acidity of 40 μEq of H^+/g of food (suspension in saline solution), whereas the high-fat chow has a pH of 6.0 and acidity of 15 μEq of H^+/g of food (suspension in saline solution), suggesting that the decrease in cilia numbers observed compared with the control solution (pH 7.4, 0.3 μEq of H^+/ml) was due to change in acidity. Interestingly, the numbers of cilia observed *ex vivo* were similar to those of mice fed with both diets for 0.5 h (*in vivo* standard chow 0.67±0.03 and high-fat chow 0.70±0.04 cilia/gland *vs. ex vivo* standard chow 0.66±0.04 and high-fat chow 0.71±0.05 cilia/gland). When we analyzed the antrum, we found a significant decrease in the cilia numbers with both chow formulations (Fig. 4*D*). The decrease observed with standard chow was significantly lower than that observed with high-fat chow (Fig. 4*D*). Similar to what we observed in the corpus, the cilia numbers in the *ex vivo* experiments with chow were similar to those of mice fed for 0.5 h (*in vivo* standard chow 0.82±0.02 and high-fat 0.96±0.05 cilia/gland *vs. ex vivo* standard chow 0.70±0.05 and high-fat chow 0.90±0.04 cilia/gland). From these experiments, we concluded that cilia presentation in the stomach responds to different signals: in the corpus, ciliated cells respond to distension and changes in gastric acidity, whereas in the antrum, cilia respond to luminal components in food.

To test directly whether cilia participate in the func-

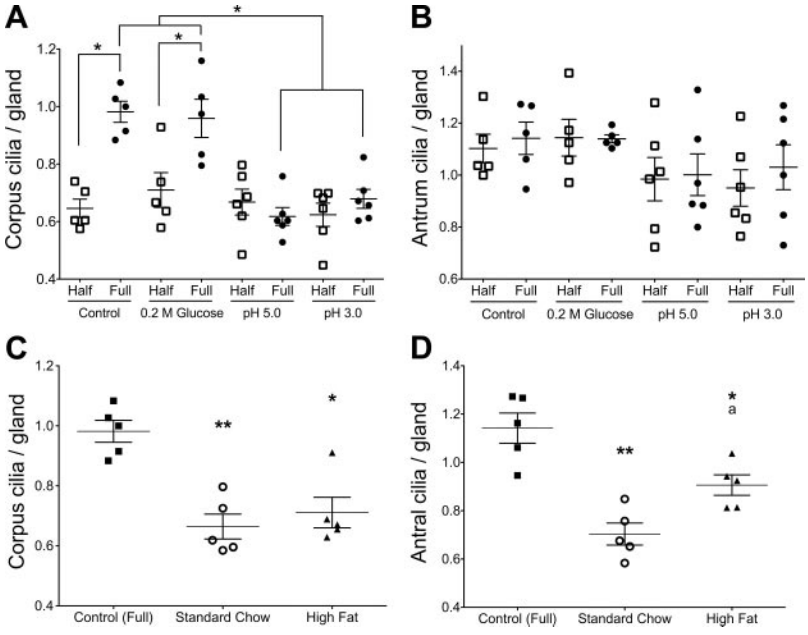

Figure 4. Number of ciliated cells in the antrum is regulated by food. *A*, *B*) Stomach corpi (*A*) and antra (*B*) were analyzed *ex vivo* for cilia presence when distended to their half (half) or maximal (full) volumetric capacity by filling with Krebs' solution (control) supplemented with glucose or with modified pH. *C*, *D*) *Ex vivo* stomachs were also filled to full capacity with homogenized standard chow or high-fat chow, and cilia in the corpi (*C*) and antra (*D*) were quantified. *$P \leq 0.05$, **$P \leq 0.01$ *vs.* control; [a]$P \leq 0.05$ *vs.* standard chow.

TABLE 3. *Numbers of endocrine cells presenting cilia in the* Ift88$^{-/fl}$ *mice stomach*

Cell type and location	Control mice, $n = 6$			*Ift88*$^{-/fl}$ *mice, n = 4*			Cilia loss (%)
	Ciliated cells	Total cells counted	%	Ciliated cells	Total cells counted	%	
G cells							
Antrum	1085	1311	82.8	266	1869	14.2	68.6
X cells							
Antrum	283	714	39.6	23	198	11.6	28.0
Corpus	716	873	82.0	162	2361	6.9	75.1
D cells							
Antrum	661	866	76.3	54	346	15.6	60.7
Corpus	1117	1475	75.7	217	1953	11.1	64.6

tion of gastric endocrine cells, we compared the feeding responses in mice with dysfunctional cilia (*Ift88*$^{-/fl}$) with those of their littermate controls. From our previous observation that the numbers of cilia in the antrum and gastrin secretion were inversely correlated, we predicted that deleting this ciliary transport protein and disrupting ciliary function would result in an increase of gastrin gene expression and secretion. At 4.5 mo of age, the *Ift88*$^{-/fl}$ mice had significantly lower *Ift88* mRNA expression (Supplemental Fig. S3*A*), and the numbers of ciliated cells diminished in both the antral and corpus epithelia (**Table 3**; Supplemental Fig. S3*B*), whereas the numbers of stromal cilia were unaltered. G and D cells lost cilia in similar proportions. Nonciliated X cells were significantly more numerous in the corpus than in the antrum (Table 3). Collectively, *Ift88*$^{-/fl}$ mice had 52 and 70% fewer ciliated cells in the antrum and corpus, respectively.

The stomach of *Ift88*$^{-/fl}$ mice at age 4.5 mo was histologically normal (Supplemental Fig. S3*C*), suggesting that cilia are not required for epithelial cell development and differentiation. When unfed, the *Ift88*$^{-/fl}$ mice showed higher (not significant) basal levels of plasma gastrin, and they reached ~4 times the levels of plasma gastrin than control mice did after feeding of standard chow for 1 h (**Fig. 5*A***). Similar levels of plasma

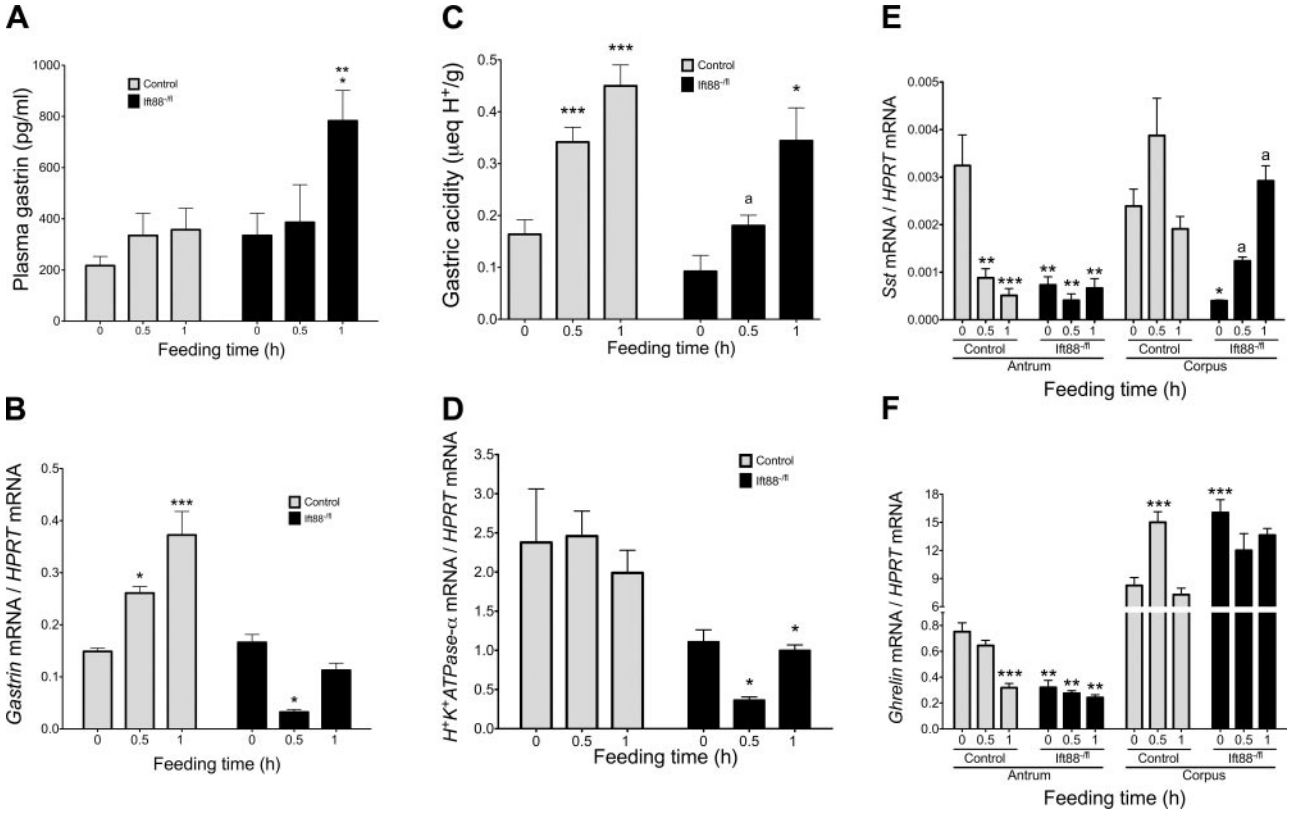

Figure 5. Elevated plasma gastrin in mice with dysfunctional cilia. *A*) Gastrin release quantified in *Ift88*$^{-/fl}$ (solid bars) mice compared with littermate control (shaded bars) mice when unfed overnight (0 h) and then allowed access to standard chow for 0.5 and 1 h. *B*) *Gastrin* gene expression in control and *Ift88*$^{-/fl}$ mice. *C–F*) Acid secretion (*C*), H^+/K^+-*ATPase* α-subunit (*D*), *Sst* (*E*), and *ghrelin* (*F*) mRNA expression. $n = 6$ control and $n = 4$ *Ift88*$^{-/fl}$ mice/time point. Data are presented as means ± SE. $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, *vs.* time 0 h; $^aP \leq 0.01$ *vs.* controls at same time point.

gastrin were observed in control mice only at 2 h (Fig. 2*B*). *Gastrin* mRNA increased consistently during the first hour of food intake in control mice (Fig. 5*B*, shaded bars). However, *gastrin* mRNA did not increase in the *Ift88*$^{-/fl}$ antra during feeding (Fig. 5*B*, solid bars). Because cilia numbers decreased in the antra of control mice with food intake, our observations suggested that cilia on G cells might participate in mechanisms that modulate gastrin gene expression and gastrin release.

We expected that the significantly higher level of circulating plasma gastrin in *Ift88*$^{-/fl}$ mice at 1 h would induce higher acid secretion. However, *Ift88*$^{-/fl}$ mice failed to achieve the same gastric acid content as the control mice (Fig. 5*C*). Because acid-producing parietal cells do not exhibit cilia, the effect observed in the *Ift88*$^{-/fl}$ mice suggested that the alteration in endocrine cell function affects gastric physiology beyond gastrin secretion. Indeed, *Ift88*$^{-/fl}$ mice had lower expression of the proton pump H^+/K^+-*ATPase* α-subunits (Fig. 5*D*) and β-subunits (Supplemental Fig. S3*D*).

Because D cells are also ciliated, Sst regulates gastrin and ghrelin (12, 13, 17), and it has been reported that the secretion of Sst in the stomach is induced by acid, gastrin, and cholecystokinin (49–51), we then analyzed *Sst* gene expression. The basal level of *Sst* expression in the corpus and antrum of the *Ift88*$^{-/fl}$ mice was significantly lower than that of controls (Fig. 5*E*). In the antrum, *Sst* mRNA was regulated by food in the control mice, but that effect was not observed in the antra of *Ift88*$^{-/fl}$ mice (Fig. 5*E*). In the corpus, *Sst* mRNA did not change significantly in the control mice (Fig. 5*E*). However, in the *Ift88*$^{-/fl}$ mice, *Sst* expression increased as a function of feeding, achieving levels equal to those of control mice at 0.5 h. In the antrum of control mice, the decrease in *Sst* mRNA with food intake correlated to an increase in *gastrin* expression. However, that was not the case for the *Ift88*$^{-/fl}$ mice, for which the increase in *Sst* mRNA was observed in the corpus and appeared to correlate with gastrin release. Our observation in the *Ift88*$^{-/fl}$ mice corpus supports the notion that Sst in the stomach is regulated by acid and gastrin.

When mice were given access to food, *ghrelin* expression in the antra of control mice decreased with time (Fig. 5*F*). In the *Ift88*$^{-/fl}$ antrum, basal *ghrelin* levels after food withdrawal were at least 50% lower than those in control mice, and no change was observed with feeding (Fig. 5*F*). In the corpi of control mice, *ghrelin* expression increased transiently at 0.5 h (as did the number of ciliated cells; Fig. 2*A*) and then returned to basal levels at 1 h, whereas in the *Ift88*$^{-/fl}$ mouse corpus, no significant changes were observed (Fig. 5*F*). We concluded that primary cilia are required to modulate gastric expression of *ghrelin*. In summary, loss of cilia function by deletion of *Ift88* dramatically affected *gastrin*, *ghrelin,* and *Sst* expression in the antrum and accelerated gastrin secretion. In the corpus, acid secretion was delayed and correlated with lower levels of the H^+/K^+-*ATPase* enzyme expression.

Although no morphological changes were observed in the *Ift88*$^{-/fl}$ stomachs at 4.5 mo of age (Supplemental Fig. S3*C*), considering the significant alterations in endocrine cell function and acid secretion in these mice, we hypothesized that they would develop gastric pathology associated with hypergastrinemia. After 8 mo of age, the stomachs of the *Ift88*$^{-/fl}$ mice showed mucous cell hyperplasia in the corpus (**Fig. 6*A***), remained hypergastrinemic (Fig. 6*B*), and were hypochlorhydric (Fig. 6*C*). The corpus showed an expanded TFF2-positive population (Fig. 6*D*) and extensive expansion of foveolar (pit) cells, as evidenced by staining with UEA-1 (Fig. 6*E*), indicating mucinous metaplasia with foveolar hyperplasia. Mild inflammation was observed in 5 of 11 mice. Consistent with known models of hypergastrinemia (14, 52, 53), the number of G cells in these mice was also increased (Supplemental Table S1). No differences in any other cell types were found.

To further confirm that the changes observed in the stomachs of the *Ift88*$^{-/fl}$ mice were indeed related to the loss of cilia function, we analyzed the stomachs of mice with conditional deletion of the kinesin subunit *Kif3a* (*Kif3a*$^{-/fl}$). As expected, the 7-mo-old *Kif3a*$^{-/fl}$ mice were hypergastrinemic (Supplemental Fig. S4*A*) and hypochlorhydric (Supplemental Fig. S4*B*) and developed foveolar hyperplasia (Fig. 6*F*), similar to the *Ift88*$^{-/fl}$ mice. Although parietal cells were still present in the mucosa (Fig. 6*G*), they did not secrete acid as in control mice. In the *Kif3a*$^{-/fl}$ mice, we also observed an increase in the number of G cells, and no differences in X or D cells compared with controls (Supplemental Table S2). These results confirmed that the disruption of cilia in the gastric epithelium results in endocrine cell dysfunction, leading to hypergastrinemia and the development of gastric metaplasia.

DISCUSSION

Cilia are sensory structures found in most tissues, although their numbers and distribution vary greatly with tissue type. Information on the presence of cilia in the GI tract is limited to reports of their transient presence during the development of the human and mouse esophagus (44, 54), the presence of multiciliated cells in gastric metaplasia (55, 56), and cells with a single cilium reported in the quail antrum (57). Here we demonstrated the presence of cells with a single cilium (primary cilia) in the normal adult mammalian stomach and identified endocrine cells as the main ciliated cell lineage. Interestingly, we did not find ciliated cells in the intestinal or colonic epithelium. Although there are gastrin-, ghrelin-, and Sst-expressing cells in the proximal duodenum, the lack of cilia in intestinal endocrine cells suggests functional differences in the signals regulating gastric *vs.* intestinal endocrine cell lineages.

Endocrine cell function in the stomach is tightly associated with food intake and acid secretion. To define the role of cilia in endocrine cells, we analyzed the gastric mucosa during food intake. We observed a

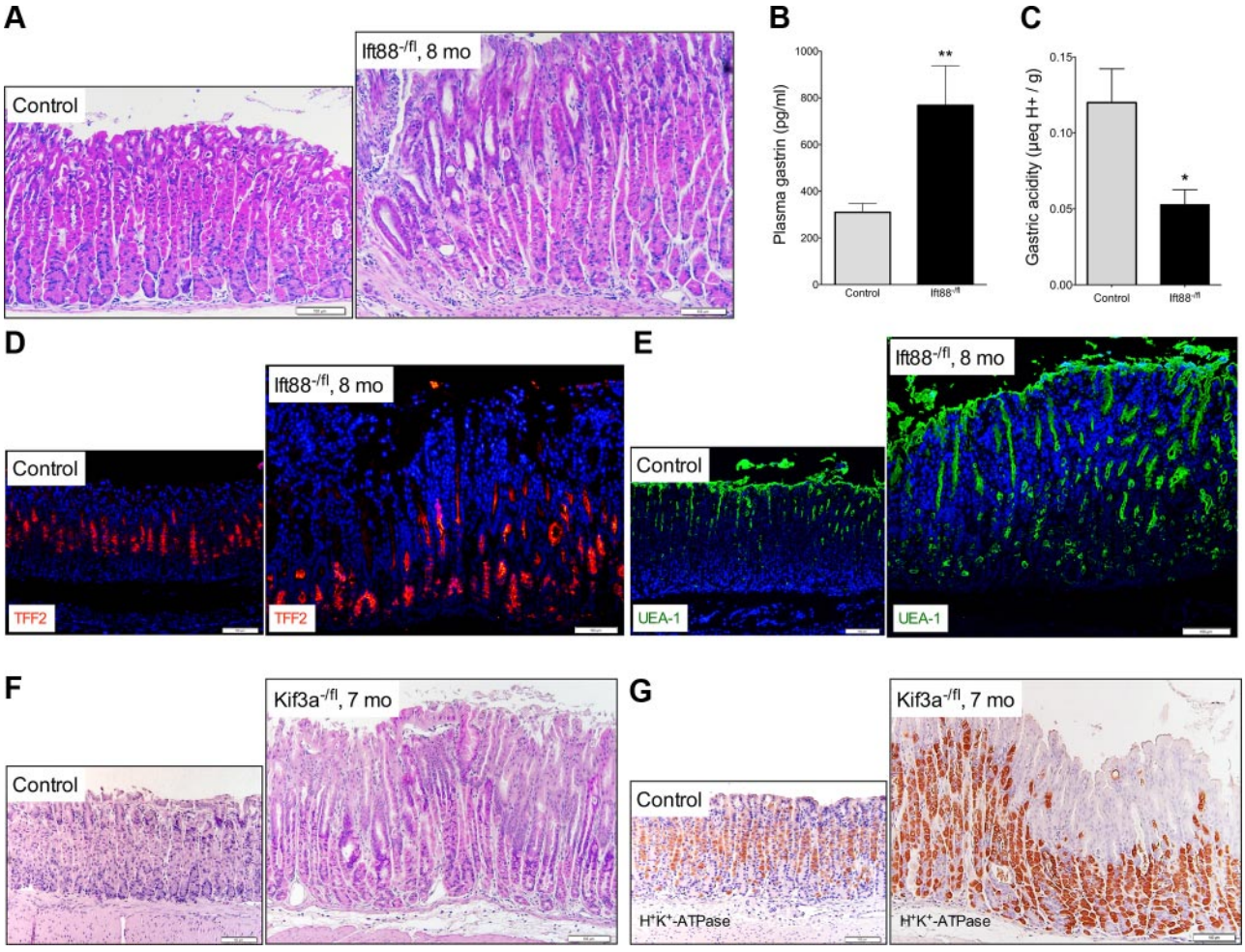

Figure 6. Cilia-deficient mice developed gastric hyperplasia. *A*) Hematoxylin and eosin stains of 8-mo-old control and *Ift88*$^{-/fl}$ mice. *B*, *C*) Plasma gastrin levels (*B*) and gastric acidity (*C*) were determined in the same mice. *D*, *E*) Mucous neck TFF2-positive cell population (*D*) was not as expanded as the UEA-1 population (*E*), demonstrating development of foveolar hyperplasia in 8-mo-old *Ift88*$^{-/fl}$ mice. *F*) Hematoxylin and eosin stains of stomachs from mice harboring deletion of the ciliary kinesin subunit *Kif3a* (*Kif3a*$^{-/fl}$) at 7 mo of age. *G*) Immunostaining of the *Kif3a*$^{-/fl}$ mice corpus with anti-H^+/K^+-ATPase antibody to identify parietal cells. *Ift88* control, $n = 10$; *Ift88*$^{-/fl}$, $n = 11$; *Kif3a* control, $n = 6$, *Kif3a*$^{-/fl}$, $n = 9$. Data are presented as means ± SE. $^*P \leq 0.05$, $^{**}P \leq 0.01$ *vs.* controls.

significant decrease in the number of ciliated cells in the antrum of fed mice compared with that in mice unfed overnight. However, the major changes in the number of cilia occurred during the first 2 h after food intake. There were no differences in the number of gastrin-, ghrelin- or Sst-expressing cells in overnight-unfed compared with fed mice. Because differentiated cells in the gastric epithelium do not undergo mitosis and their life span is ~2 mo (47), we concluded that cilia presentation in endocrine cells in the stomach is dynamic and responds to food as opposed to the cell cycle. Using a reporter mouse for *gastrin* expression, Takaishi *et al.* (16) found a significant decrease in the number of cells positive for the *gastrin* reporter under nonfeeding conditions. In this study, we also observed lower *gastrin* gene expression and plasma gastrin in overnight-unfed mice. These results support the observation that G cells do not release gastrin until appropriate signals are detected (13).

Our results reported here suggest that primary cilia mediate the signaling for gastrin release. Gastrin expression is regulated by diverse signals including nutrients, hormones, neurotransmitters, pH, and stomach distension (46). Although there are numerous studies regarding the regulation of *gastrin* gene expression and secretion, there remains a paucity of information on the signals regulating *ghrelin* and *Sst* expression in the stomach. In our study, feeding mice standard chow triggered a decrease in the number of antral cilia 30 min before the peak in gastrin and acid secretion. In contrast, feeding mice a high-fat diet did not stimulate the expected decrease in antral cilia numbers. Gastrin levels achieved during the first 4 h of feeding high-fat chow were significantly lower than those obtained with standard chow. However, acid secretion remained elevated in mice fed a high-fat diet. When mice were fed a high-fat diet for 3 wk, their basal antral cilia number decreased, and circulating gastrin levels were higher, but their basal gastric acid levels were lower than those in mice fed standard chow. Different studies have

analyzed gastrin secretion in response to a high-fat diet. A study in healthy male volunteers showed that a fat meal induced a significant rise in serum gastrin that peaked 30 min after food ingestion, and its maximum level was maintained for 60 min (58). In Mongolian gerbils, long-term feeding with a high-fat diet resulted in gastrin levels higher than those of gerbils fed regular or high-protein chow (59). However, feeding rats a high-fat diet for 2 wk resulted in a decrease in serum gastrin, and an attenuation in gastrin levels when they were refed for 60 min compared with when they were fed a regular diet (60). In our studies, we observed less gastrin release during the first hours after high-fat food intake that correlates with the prior study performed in rats. The resulting elevated gastrin levels after 3 wk of high fat intake correlates with the observation made in gerbils. However, the experimental design of each study makes it difficult to generalize the effect of a high-fat diet on gastrin release. In addition, studies on the effect of a high-fat diet on acid secretion are also scarce.

When stomachs were studied *ex vivo*, we observed that cilia presentation responded to different stimuli. In the corpus, cilia were affected by distension and pH, whereas in the antrum, they were affected only by presence of food. Numerous studies on gastrin release have used isolated antra, isolated stomachs, or G-cell preparations (46, 61), suggesting that gastrin release is regulated mainly by luminal stimuli. In a study with volunteers trained for sham feeding (masticating without swallowing food), Hildebrand *et al.* (62) showed that sham feeding induced gastric acid secretion, but not plasma gastrin. Our observations that antral cilia diminished only in the presence of food and that their reduction was associated with gastrin release suggest an important role for cilia in sensing luminal signals in the stomach.

When we analyzed mice with dysfunctional cilia (conditionally deleted *Ift88* and *Kif3a*), we observed that the levels of circulating gastrin remained elevated compared with those in control mice, even when *gastrin* and *Sst* mRNA expression were significantly lower than that in their littermate controls. Sst has been reported to inhibit both gastrin gene expression and secretion (12, 13) and that gastrin regulates *Sst* expression (50). However, in cilia-deficient mice antrum, *Sst* mRNA was lower than in control antrum, and we did not observe a rise in *gastrin* mRNA, whereas circulating gastrin remained elevated. Thus we concluded that the loss of cilia function acts at the level of gastrin release as opposed to regulating *gastrin* mRNA and might be independent of Sst control.

Cilia-deficient mice showed higher levels of *ghrelin* mRNA in the corpus compared with those in controls, whereas *Sst* was lower. It has been reported that Sst suppresses ghrelin secretion at the protein level (63) but that no changes in *ghrelin* mRNA expression were observed *in vitro* (64), suggesting that Sst regulation of ghrelin also occurs at the post-translational level and that lower *Sst* might not account for the deregulation of *ghrelin* expression we observed in cilia-deficient mice. Thus, Sst might not trigger the signal transmitted by the cilium on the X cells. In addition, whereas the Sst receptor 3 specifically localizes to primary cilia in endocrine pancreatic cells (30), the Sst receptors 2 and 5 are the predominant forms present in the stomach (65, 66). To our knowledge, there are no reports on localization to cilia of these two Sst receptor subtypes. There are other possible mechanisms for ghrelin regulation. X cells express taste receptors (67) and might sense the gastric luminal contents (68), *e.g.*, food. In addition, the amino acid receptor G-protein-coupled receptor family c group 6 member A (GPRC6A), along with the calcium-sensing receptor and the taste receptor subunit T1R3, has been reported recently to be on endocrine cells of the antrum (69). Whether these receptors localize to the cilia on gastric endocrine cells remains to be determined.

Acid secretion is important for food digestion, microbial activity, and gastric homeostasis. Loss of acid secretion is one of the first steps for development of gastric metaplasia. Here we showed that cilia-deficient mice failed to achieve normal levels of acid secretion, exhibited elevated plasma gastrin, and developed foveolar hyperplasia with time. The mechanism for this observation is still unknown. Nevertheless, we showed decreased expression of the proton pump H^+/K^+-ATPase-α and -β subunits in cilia-deficient mice. However, the development of gastric hyperplasia ad metaplasia associated with endocrine deregulation and hypochlorhydria is a chronic event and was not evident in the cilia-deficient mice until after 7 mo of age. Possible explanations for the protracted time course include endocrine deregulation in the stomach that might result in epigenetic changes in *Sst* expression (70), subsequently leading to reduced control of acid secretion.

There is no information linking gastric metaplasia to ciliopathies, probably due to the severity of the phenotypes found in other organs (71) or the lack of symptoms that characterize most gastric mucosal changes. Obesity, however, is characteristic of two ciliopathies, Bardet-Biedl syndrome (72) and Alström syndrome (73). Gastric ghrelin and gastrin have been shown to affect glucose homeostasis and food intake, suggesting a possible role for gastric endocrine cells in the development of obesity, particularly in the context of ciliopathies. Recently, Kentish *et al.* (74) reported that signaling of gastric distension by vagal afferents was reduced after food withdrawal and feeding of a high-fat diet for 12 wk, which might be associated with changes in acid secretion observed in our feeding experiments. They also reported that food withdrawal and a high-fat diet also enhanced the ghrelin orexigenic effect, suggesting that alterations in gastric physiology play a significant role in the development of obesity.

In summary, our results implicate cilia in the corpus and antrum as transducers of different signals: on the antral cells, cilia participate in the endocrine response to food particularly *via* gastrin secretion, whereas in the

corpus, cilia respond to acid and distension, but their role is still unclear. Overall, our study demonstrates that gastric endocrine cells sense food through primary cilia, and such structures are necessary for maintaining gastric homeostasis.

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