Upper endoscopy and biopsy

During endoscopy, biopsy specimens were obtained from the antrum, body and fundus for histological studies. Specimens taken from the fundus were used to measure the mRNA of preproghrelin, TRPV1, GDNF and NGF.[16]

The baseline *H. pylori* infection status was determined based on modified Giemsa staining, culture and rapid urease test (CLOtest; DeltaWest, Bentley, Australia). If one of these invasive *H. pylori* tests was positive, the patient was diagnosed with *H. pylori* infection. Concerning histology, the degree of atrophy and intestinal metaplasia were recorded using the updated Sydney scoring system (0: none, 1: slight, 2: moderate, and 3: marked).[26]

Measurement of preproghrelin and nociception-related gene expression

Biopsy specimens from the gastric fundic mucosa were stored in liquid nitrogen and total RNA was extracted from fundus with use of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were diluted to a final concentration of 0.5 mg/mL in RNase-free water and stored at -80 °C. Synthesis of the complementary DNA was performed with 1 lg of total RNA with M-MLV reverse transcriptase (Invitrogen). The thermal cycling parameters for the reverse transcription reaction were 10 min at 65 °C, 50 min at 37 °C and 15 min at 70 °C. Real-time PCR and determination were performed with SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) and a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used: preproghrelin forward primer, GGG CAG AGG ATG AAC TGG AA; preproghrelin reverse primer, CCT GGC TGT GCT GCT GGT A; TRPV1 forward, 5′-GAGTTTCAGGCAGACA CTGGAA-3′; TRPV1 reverse, 5′-CTATCTCGAGCACTTGCCTCTCT-30; GDNF forward, 5′-CTTGGGTCTGGGCTATGAAAC-30; GDNF reverse, 5′-CAAAGGCGATGGGTCTGC-3′; NGF forward, 5′-AGCAAGCGGTCATCATCC-3′; and NGF reverse, 5′-GTGGCGGTG

GTCTTATCC-3′. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer, AGG TGA AGG TCG GAG TCA; and GAPDH reverse primer, GGT CAT TGA TGG CAA

CAA. The GAPDH gene was used as an endogenous reference. The initial denaturation step at 95 °C for 10 s was followed by 40 cycles of denaturation for 5 s at 95 °C and annealing/extension for 33 s at 55 °C for GAPDH and at 58 °C for preproghrelin. Relative expression of target genes was normalized by division of the target Ct values by the endogenous Ct values.

Measurement of plasma ghrelin level

Blood samples were obtained after an 8-h overnight fast, immediately transferred to chilled polypropylene tubes containing Na_2EDTA and aprotinin and centrifuged at 4 °C. One tenth of the volume of 1 N HCl was added to the separated plasma. Desacyl ghrelin and acyl ghrelin were measured by use of ELISA kits to active ghrelin and desacyl ghrelin (SCETI CO., LTD., Tokyo, Japan). The intra-assay and interassay coefficients of variation were 6.5% and 9.8% for acyl ghrelin, respectively, and 3.7% and 8.1% for desacyl ghrelin, respectively. All tests were performed at least in duplicate.

Supplementary Table 1 Dyspepsia symptoms, stool consistency and bowel movement according to the presence or absence of gynecologic surgery in the female functional dyspepsia group

Symptoms	Without abdominal surgery (*n* = 23)	With abdominal surgery (*n* = 25)[1]	*P* value
Abdominal pain (frequency)[2]	3.0 ± 0.4	4.2 ± 0.2	0.070
Abdominal pain (Severity)[2]	3.0 ± 0.2	3.3 ± 0.2	0.046
Early satiation (mean ± SE)	3.6 ± 0.3	3.8 ± 0.7	0.616
Postprandial fullness	2.7 ± 0.3	3.1 ± 0.2	0.279
Epigastric burning/pain	3.0 ± 0.1	3.3 ± 0.3	0.195
Bloating	2.4 ± 0.3	2.3 ± 0.7	0.665
Nausea	1.3 ± 0.3	2.5 ± 0.4	0.046
Vomiting	0.4 ± 0.2	0.9 ± 0.1	0.181
BSFS	4.0 ± 0.2	4.1 ± 0.3	0.466
Number (per week)	4.1 ± 0.3	4.5 ± 0.3	0.362

[1]25 underwent abdominal surgery (12 hysterectomy or oophorectomy, 13 Cesarean section); [2]Pain was not restricted to the epigastric area. BSFS: Bristol stool form score (from 1 = very hard to 7= watery); FD: Functional dyspepsia; SE: Standard error.