Published in final edited form as:

Gastroenterology. 2011 September ; 141(3): 854–863. doi:10.1053/j.gastro.2011.05.044.

Estrogen Regulation of Duodenal Bicarbonate Secretion and Sex-Specific Protection of Human Duodenum

Biguang Tuo^{1,*}, Guorong Wen¹, Jinqi Wei², Xuemei Liu¹, Xue Wang¹, Yalin Zhang¹, Huichao Wu¹, Xiao Dong³, Jimmy Y.C. Chow³, Volker Vallon^{3,4}, and Hui Dong^{1,3,*}

¹ Department of Gastroenterology, Affiliated Hospital, Zunyi Medical College, Zunyi, China

² Department of Gastroenterology, the Fifth Affiliated Hospital, Sun Yat-Sen University, Zhuhai, China

³ Department of Medicine, University of California, San Diego, California, USA

⁴ VA San Diego Healthcare System, San Diego, California, USA

Abstract

BACKGROUND & AIMS—The reason that women have a lower prevalence of duodenal ulcer is not clear. We investigated whether estrogen regulates human duodenal bicarbonate secretion (DBS) and whether this process accounts for sex differences in the prevalence of duodenal ulcer.

METHODS—We performed an epidemiological study to correlate duodenal ulcer prevalence with sex and age. Proximal DBS was measured from healthy subjects. Estrogen receptor expression was examined in human duodenal mucosa by immunoblot and immunohistochemical analyses.

RESULTS—Among women, the prevalence of duodenal ulcer was significantly lower than among men. The reduced prevalence was greatest among premenopausal women (20–49 years), who were 3.91–5.09-fold less likely to develop duodenal ulcers than age-matched men; the difference was reduced to ≤ 1.32 -fold among subjects 60 years or older. Premenopausal (20–29 years), but not post-menopausal (60–69 years) women, had significantly higher basal and acid-stimulated DBS than the age-matched men. Basal and acid-stimulated DBS in premenopausal women (20–29 years) were significantly higher than in post-menopausal women (60–69 years), whereas there were no significant differences in basal or acid-stimulated DBS between men that were 20–29 years old or 60–69 years old. Serum levels of estradiol changed in parallel with basal

© 2011 The American Gastroenterological Association. Published by Elsevier Inc. All rights reserved.

*Correspondence: Hui Dong, MD, PhD: Division of Gastroenterology, Department of Medicine, 9500 Gilman Drive La Jolla, CA 92093, h2dong@ucsd.edu Tel: 858-534-9862 Fax: 858-534-3338 or Biguang Tuo, MD, PhD: Department of Gastroenterology, Affiliated Hospital of Zunyi Medical College, 149 Dalian Road, Zunyi 563003, China. tuobiguang@yahoo.com.cn Tel: +86-852-8609205. Fax: +86-852-8609205.

Disclosures: No conflicts of interest exist

Author contributions:

BT and HD: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; administrative, technical, or material support; study supervision.

GW: acquisition of data; administrative, technical, or material support.

JX, XL, XW, YZ, HW, XD: acquisition of data.

JYCC and VV: critical revision of the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and acid-stimulated DBS during the physiological menstrual cycle in premenopausal women. 17 β -estradiol-stimulated DBS was independent of age or sex. Estrogen receptors- and - were detected on plasma membrane and in cytosol of human duodenal epithelial cells.

CONCLUSIONS—Estrogen regulates human DBS, which could reduce the risk for duodenal ulcer in women and contribute to sex differences in prevalence of duodenal ulcer.

Keywords

intestine; intestinal ER; menopause; sex hormone

Introduction

Peptic ulcers, especially duodenal ulcer, are common alimentary diseases with high prevalence and inherent economic costs to the world.^{1, 2} Duodenal ulcer occurs when the balance between aggressive and defensive factors is disrupted. Although the role of aggressive factors in the pathogenesis of duodenal ulcer has been extensively studied, the role of defensive factors and their regulation are poorly understood. Duodenal mucosal bicarbonate secretion (DBS) plays an important role in duodenal mucosal protection against acid-induced injury.^{3, 4} Patients with duodenal ulcer have significantly diminished proximal DBS, compared with healthy volunteers⁵, suggesting that diminished DBS contributes to the pathogenesis of duodenal ulcer.

Duodenal ulcer occurs more often in men than in women. The male/female ratio in the incidence of duodenal ulcer is 1.9:1 in USA, 2.2:1 in Europe and 3.6:1 in China.^{2, 6, 7} Interestingly, pregnant women or women taking contraceptives (estrogenic/progesterone compounds) are less likely to develop duodenal ulcer.⁸ Moreover, a more serious course of duodenal ulcer in postmenopausal women than those during premenopausal period⁹, suggests that sex difference in duodenal ulcer is likely due to female sex hormones. Although estrogens are known to protect against cardiovascular diseases¹⁰, cerebral damage and mortality in women¹¹, little is known about their protective role in gastrointestinal tract.

Estrogen was shown to prevent peptic ulcer in animals^{12, 13}, but the mechanisms remain unclear. Recently, we reported for the first time that¹⁴: 1) the basal and acid-stimulated DBS were higher in young female than age-matched male mice; 2) acid-stimulated DBS could be abolished by estrogen receptor (ER) antagonists in mice; 3) 17 β -estradiol dose-dependently stimulated DBS in mice *in vitro* and *in vivo*; and 4) ER and ER were expressed in murine duodenal epithelium of both sexes. Since DBS is important to protect duodenum against acid-induced injury, sex-associated differences in DBS could explain the lower duodenal ulcer occurrence in women. Therefore, we examined if human DBS is regulated by estrogen and if this regulatory process is associated with lower duodenal ulcer occurrence in premenopausal women. Our findings substantiate the novel concept that estrogen regulates human DBS, which may contribute to the sex differences observed in duodenal ulcer occurrence.

Patients and Methods

Data source of epidemiological study

This study includes all the patients with dyspepsia who underwent upper gastrointestinal endoscopy from January 2000 to December 2007 in the Endoscopic Units of the Affiliated Hospital of Zunyi Medical College and the Zunyi Hospital located in Southwest China. All patients completed a detailed questionnaire. Patients younger than 20 years or with previous history of peptic ulcer were excluded. Duodenal ulcer was defined as the presence of active,

healing, or scarring ulcer. Two biopsies were taken from the gastric antral mucosa of all patients to test for *Helicobacter pylori* (*H. pylori*) infection by rapid urease test. *H. pylori*-negative samples were further submitted for histologic examination for *H. pylori*, or C14 urea breath test. Patients were considered *H. pylori*-infected if at least one test was positive. A total of 64,385 patients were included in this study.

Measurement of proximal DBS in humans

Subjects—The study was approved by the Human Subjects Committee in Affiliated Hospital of Zunyi Medical College, Zunyi, China. Healthy Chinese subjects of both sexes, age 20–29 and 60–69 years, without history of gastrointestinal disease, *H. pylori* infection, or other medical disease were selected for the study. *H. pylori* infection was examined by ¹⁴C urea breath test and serum *H. pylori*-Ig G assay with ELISA. Subjects were considered non-*H. pylori*-infected only when results of both tests were negative. None of the healthy volunteers had ingested oral contraceptive pills, non-steroid anti-inflammatory drugs (NSAIDs), alcohol, and cigarette smoking a week before the study. Each subject has given a written informed consent.

Functional isolation of the proximal duodenum and measurement of DBS—

Volunteers were divided into two age groups (20–29 and 60–69 years). The experiments were performed at random in each group. The proximal duodenal segment was functionally isolated as described previously⁵ with minor modifications. Briefly, after overnight fasting, cimetidine (300 mg) was given intramuscularly to suppress gastric acid secretion and to prevent entry of gastric acid into the isolated test segment of duodenum. A double-lumen nasogastric tube was inserted into the stomach to perfuse phenol red and aspirate gastric juice to prevent gastric juice from entering the duodenum. Next, a duodenal tube with five channels and three balloons (Kangling Corp, Zhejiang, China) (Figure 1) was inserted into the stomach via nostril, and the tube was positioned using special placing-clips for duodenal tube under gastroscopy. The duodenal bulb balloon (second balloon) was inflated with 25 ml of air. Gentle traction was applied to the tube to anchor the balloon immediately below the pylorus. The gastric balloon (first balloon) was then inflated with 35 ml of air to further occlude the pylorus region. The duodenal segment was flushed with NaCl solution (154 mM) for 15 minutes to remove residual gastric or duodenal juice. Then the third balloon was inflated with 25 ml of air, to functionally isolate 4 cm of the proximal duodenal segment. This segment was then perfused with NaCl solution (154 mM) at 2 ml/min using a syringe pump. The effluent was collected from the distal portion of the test segment by gravity drainage in a sample cup for every 15 min. Basal DBS was measured in four 15-min periods. Subsequently, the isolated segment was exposed to 10 ml HCl (100 mM), 10 ml 17 β -estradiol (0.1mg/ml) (Sigma Corp, USA), or vehicle control for 10 min to test for effects of duodenal acidification or estrogen on DBS. Then the third balloon was deflated. The test segment was gently flushed with NaCl (154 mM) for 5 min to remove any residual acid or estradiol in the isolated duodenal segment. The third balloon was inflated again. HCO₃⁻ secretion was measured for an additional four to five 15-min periods. HCO₃⁻ concentration in the effluent was measured in triplicate immediately upon collection by back titration. Concentrations of phenol red and trypsin were measured in each effluent sample to confirm no gastric or distal duodenal juice in the isolated segment of duodenum.

To further investigate the physiological role of endogenous estradiol in DBS regulation, basal and acid-stimulated DBS and serum estradiol concentration were measured at the same time in 8 healthy premenopausal female volunteers (20–29 years) during their preovulatory phase (2–3 days before ovulation) or premenstrual phase (2–3 days before menses). The premenstrual and preovulatory phases were determined using menstrual cycle information provided by subjects and ovarian follicle visualization using ultrasound type B. Serum

estradiol concentration was measured by chemiluminescent enzyme immunoassay (IMMULITE 2000 Estradiol, DPC, USA).¹⁵

Expressions of estrogen receptors by Western blot analysis

Proximal duodenal mucosal tissues taken by biopsy under gastroscopy in male and female volunteers (20–29 years of age) were snap-frozen and stored in liquid nitrogen. Tissue samples were homogenized in 500 µl of lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton-100, 0.5 mM EDTA, 1 mM PMSF). Protein was normalized (50 µg/ml) and was separated by 12% SDS-PAGE, transferred to PVDF membranes, and probed with primary antibodies, anti-ER (Santa Cruz, 1:200 dilution), anti-ER (Santa Cruz, 1:300 dilution), and anti-Actin (Santa Cruz, 1:500 dilution) at 4°C overnight. This was followed by alkaline phosphatase-conjugated goat anti-rabbit IgG incubation for 1 hour at room temperature. Immunoreactive proteins were visualized by enhanced chemiluminescence (Fivephoton Biochemicals, San Diego, CA).

Expression of estrogen receptors by immunohistochemistry analysis

Proximal duodenal mucosal tissue taken by biopsy under gastroscopy in male and female volunteers (20–29 years of age) were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. The tissue block was cut in 5 µm sections, deparaffinized, and heated in citrate buffer (10 mmol/L citric acid; pH 6.0) using a microwave oven (750 W) for 10 min before immunostaining. Endogenous peroxidase activity was quenched by 3% hydrogen peroxide in methanol for 10 min. After incubation with rabbit anti-human polyclonal ERα antibody (MC-20; Santa Cruz) or rabbit anti-human polyclonal ERβ antibody (H-150; Santa Cruz) overnight at 4°C, the sections were washed with PBS and exposed to Dako EnVision Detection Systems Peroxidase/DAB, Rabbit/Mouse (Dako) for 30 min at room temperature. Slides were rinsed in PBS, incubated with the Dako Liquid DAB Large-Volume Substrate-Chromogen System, rinsed gently with distilled water, and counterstained with hematoxylin. Coverslips were mounted using mounting media and then examined using a Leica microscope. The specificity of immunoreactivity was confirmed by using cultured HepG2 cells as negative controls (hepatocellular carcinoma cell lines, our PCR and western blot analyses confirmed that there were no expressions of mRNA and protein of ERα and ERβ) and by using nonimmune rabbit antiserum instead of the primary antibodies. Tissue of human breast carcinoma was used as positive control.

Statistical analysis

Statistical analysis was performed using the SPSS PC statistic package. Chi Square (χ^2) test and Fisher's exact probability test were applied to compare the prevalence of *H. pylori* infection and duodenal ulcer for different ages and sex. All results in DBS experiments and serum estradiol measurements are expressed as means $\pm$ SEM. Net peak DBS refers to stimulated peak responses minus basal value. Basal value refers to average of four 15-min measurements performed during 1-h. Data were analyzed by two-tailed Student t-test for paired or unpaired values as appropriate. $P < 0.05$ was considered statistically significant.

Results

Age and sex differences of duodenal ulcer prevalence in Chinese population

We conducted an epidemiological cohort study to assess the age and sex differences of duodenal ulcer prevalence in dyspeptic patients of Southwest China. The patients were divided into seven age groups using 10-year intervals. A total of 64,385 patients, 31,249 men (48.54%) and 33,136 women (51.46%), were evaluated. There were no significant differences in subject numbers and *H. pylori* infections between male and female patients in

each age group ($P>0.05$) (Figure 2A and B). Of 64,385 patients, 5,042 patients (7.83%) had duodenal ulcer. The prevalence of duodenal ulcer in men was high but similar (10.09–13.14%) in all age groups. In contrast, while the prevalence of duodenal ulcer in women under the age of 49 years (premenopausal) was low (2.18–3.36%), it markedly increased (8.32–9.11%) in women older than 60 years (postmenopausal). Men were 3.91–5.09 fold more likely to have duodenal ulcer than age-matched women in the age group of 20–49 years ($P<0.001$), and this sex difference was reduced to ≤ 1.32 fold in subjects older than 60 years ($P<0.05$ or $P>0.05$) (Figure 2C). Moreover, the male/female ratio in duodenal ulcer prevalence in patients younger than 50 years was independent of *H. pylori* infection because the ratio is similar between *H. pylori*-infected and non-*H. pylori*-infected groups (4.68 fold for *H. pylori* infection vs. 4.09 fold for non-*H. pylori* infection) (Figure 2D).

Age and sex differences of basal and acid-stimulated DBS in healthy men and women

DBS is an important defensive factor in duodenal ulcer development. Here we hypothesized that DBS is different between pre- or post-menopausal women and age-matched men. To test this hypothesis, we measured basal and acid-stimulated DBS in healthy male and female subjects in two age groups (20–29 years (premenopausal) and 60–69 years (postmenopausal)) in Southwest China. Basal DBS was stable for 60 min in all groups and was significantly higher in women than in men in the age group of 20–29 years ($P<0.05$) (Figure 3A and B). While duodenal luminal administration of vehicle control did not alter basal DBS (data not shown), 10 ml HCl (100 mM) resulted in a robust increase in DBS, which maximized within 30 min. The time course of acid-stimulated DBS was similar in both sexes. However, the net peak DBS was significantly higher in women than in men in the age group of 20–29 years ($P<0.01$) (Figure 3A and B). In contrast, both basal and acid-stimulated DBS were not significantly different between women and men in the age group of 60–69 years ($P>0.05$) (Figure 3C and D). Additionally, basal DBS was significantly lower in 60–69 years old women compared with 20–29 years old women ($p<0.05$), but there was no significant difference between men in these two age groups ($p>0.05$) (Figure 3E). Acid-stimulated net peak DBS was also significantly different in women of these two age groups ($p<0.01$), but was not affected by age in men ($p>0.05$) (Figure 3F). Overall, the age and sex differences we observed for duodenal ulcer prevalence correlated with the differences we found in DBS (Figure 2C vs. Figure 3).

Basal and acid-stimulated DBS at different stages of menstrual cycle in healthy premenopausal women

To test if endogenous estrogen plays a physiological role in the regulation of human DBS, we compared the basal and acid-stimulated DBS in preovulatory and premenstrual women. Serum estrogen level varies with female menstrual cycle and serum estradiol level is higher in preovulatory phase than in premenstrual phase.¹⁶ Eight healthy female volunteers (20–29 years) were selected for the study. As shown in Figure 4A and B, the basal and acid-stimulated DBS were significantly higher in the preovulatory phase than in the premenstrual phase ($p<0.05$). Serum estradiol concentration was $1,135 \pm 135$ pmol/L in the preovulatory phase vs. 272 ± 27 pmol/L in the premenstrual phase ($p<0.001$) (Figure 4C). We also found that: 1) the average basal DBS was 159 ± 22 vs 234 ± 26 mol/cm-h in young women (20–29 years) at premenstrual phase or preovulatory phase. The average basal DBS in heterogeneous young women in terms of menstrual status (Figure 3B) was 199 ± 25 mol/cm-h, which is between the values of premenstrual and preovulatory phases. 2) The acid-stimulated net peak DBS was 359 ± 42 vs 515 ± 45 mol/cm-h in young women at premenstrual phase or preovulatory phase. The acid-stimulated net peak DBS in heterogeneous young women in terms of menstrual status (Figure 3B) was 468 ± 32 mol/cm-h, which is again between the values of premenstrual and preovulatory phases. Taken

together, these data demonstrate that basal and acid-stimulated DBS correlate with the serum estradiol concentration during the menstrual cycle of premenopausal women.

17 β -estradiol stimulates DBS to a similar extent in healthy men and women

The above results indicate that estrogen is a strong candidate to explain sex differences in humans and age differences in women of DBS. To further strengthen this hypothesis, we assessed the effects of 17 β -estradiol on DBS in healthy male and female subjects in the age groups of 20–29 years and 60–69 years. Basal DBS in 20–29 years old women (Figure 5A and B) ($P < 0.05$) but not in 60–69 years old women (Figure 5C and D) ($P > 0.05$) was significantly higher than in age-matched men, consistent with the results above. Duodenal luminal administration of 17 β -estradiol significantly stimulated DBS in groups of all age and sex (Figure 5A and C) ($P < 0.01$), and the time course and net peak of 17 β -estradiol-stimulated DBS were similar between groups (Figure 5B and D) ($P > 0.05$). Thus, 17 β -estradiol directly stimulated DBS that is independent of sex and age, consistent with an important role of estrogen in the regulation of DBS in humans.

Expression and localization of ER in human duodenum

Since the results above indicate that estrogen is involved in the regulation of human DBS, we determined ER expression in human proximal duodenal mucosae by Western blot analysis. Both ER α and ER β proteins were expressed at similar levels in the duodenal mucosae of healthy male and female volunteers (20–29 years) (Figure 6). They were also expressed in duodenal mucosal epithelial cells (Figure 7A and B). Of note, they were mainly localized to the plasma membrane and cytosol of the villous and crypt cells in duodenal mucosa. Breast carcinoma cells and hepatic carcinoma cells were used as a positive and negative control, respectively (Figure 7C and D).

Discussion

Duodenal ulcer is a common alimentary disease. Many factors, including *H. pylori* infection, genetic and environmental factors, and immunity are likely involved in the development of duodenal ulcer. As *H. pylori* infection is currently considered an essential component for the development of duodenal ulcer¹⁷, clinicians tend to pay less attention to potential genetic factors associated with duodenal ulcer. In fact, sex differences in duodenal ulcer have been clinically observed for many years, even before the discovery of *H. pylori*¹⁸, but the underlying mechanisms are still elusive. In the present epidemiologic study, we found that the prevalence of duodenal ulcer was markedly higher in men than in women. However, there was no significant difference of *H. pylori* infection between male and female patients at different ages and the higher male/female ratio in duodenal ulcer prevalence appeared independent of *H. pylori* infection. The present study revealed that the sex differences in duodenal ulcer prevalence mainly existed between premenopausal women (20–49 years) and age-matched men. Whereas the prevalence of duodenal ulcer in men was similar and relatively high for all age groups, it was low in women under the age of 49 years, but markedly increased beyond 60 years. These results suggest that female rather than male sex hormones may determine the risk of human duodenal ulcer and explain the sex and age differences. Similarly, a previous animal study showed that female rats developed less duodenal ulceration than male rats and that ovariectomy made female rats more prone to duodenal ulceration.¹⁹ Female sex hormones also have significant antiulcer activity in both drug- and stress-induced ulcer models.¹² These studies demonstrated that female sex hormones may prevent the development of duodenal ulcer although the underlying mechanisms were unclear.

Cigarette smoking and alcohol drinking may contribute to the sex differences of duodenal ulcer in humans^{20, 21} because they are more common among men in Asia. However, the apparent age difference in sex ratios in duodenal ulcer prevalence suggests they are not the major contributors. Gastric acid secretion is similar in women and age-matched men,²² and, therefore, is also unlikely to explain the sex difference. Jon Isenberg demonstrated over 20 years ago that proximal DBS was impaired in patients with duodenal ulcer²³, and the protective role of DBS against gastric acid-induced duodenal injury has been recognized.^{3, 4} Here, we tested the hypothesis that estrogen induces DBS, which prevents duodenal ulcer development and contributes to the sex difference observed in the prevalence of duodenal ulcer. Previous studies excluded a role for progesterone because gastrointestinal epithelia appear to lack the expression of progesterone receptors^{24, 25}, and progesterone even at higher doses (0.1–1 μ M) did not induce DBS in mice¹⁴. We, therefore, investigated the role of endogenous and exogenous estrogen in DBS regulation in humans. We found that: 1) both basal and acid-stimulated DBS are almost 2-fold greater in premenopausal women (20–29 years) than age-matched men, but they are similar in postmenopausal women (60–69 years) and age-matched men; 2) both basal and acid-stimulated DBS are greater in premenopausal women than postmenopausal women, but there are no significant age-dependent differences in men; 3) the basal and acid-stimulated DBS are positively correlated with serum estradiol levels during the menstrual cycle of premenopausal women; 4) there is a negative correlation between DBS and duodenal ulcer prevalence in humans; and 5) 17 β -estradiol stimulated DBS in both male and female subjects. Similar to our previous studies in mice¹⁴, these studies strongly suggest that estrogen-mediated DBS contributes to sex differences in duodenal ulcer prevalence and is a protective factor. Since the concentration of 17 β -estradiol used in the present study is higher than its typical circulating concentrations in ovulating women, it might have other nonspecific effects, such as activation of TGR5, a metabotropic receptor that is a G-protein-coupled to the induction of adenylate cyclase²⁶. However, the specific action of 17 β -estradiol on DBS has been demonstrated previously in mice¹⁴.

Our Western blotting and immunohistochemistry analysis demonstrate that both sexes express similar levels of duodenal ER α and ER β . In accordance, local application of 17 β -estradiol stimulated DBS in all subjects independent of age and sex. Therefore, the observed sex differences of DBS are most likely due to the differences in estradiol levels in men and women rather than the different expression levels of estrogen receptors (ER) between different sexes. This notion has been supported by the finding that DBS is positively correlated with serum estradiol levels during the menstrual cycle of premenopausal women. Although both ER α and ER β have been detected in animal and human intestines^{27, 28}, our study is the first to show the protein expression and localization of ER in human duodenal epithelial cells. Of note, our immunohistochemistry study reveals that ER α and ER β were mainly localized to plasma membrane and cytosol of villous and crypt cells in human duodenal mucosa, which is consistent with a previous report that ER proteins are exclusively localized to epithelial cells in human small intestine, but not to endocrine glands and Brunner glands.²⁸ Since both villous and crypt cells are important secretory cell types in the intestine, the observed localization of ER would be consistent with their roles in regulating duodenal epithelial ion transport, including the non-genomic and more rapid actions of estrogens (as observed in studies with 17 β -estradiol application) through activation of ER in the plasma membrane and/or cytosol of these cells.²⁹ Previous studies localized ER staining in most epithelial tissues to the nucleus, with differences between the patterns for ER and ER^{30, 31}. Nevertheless, O'Mahoney et al. reported predominant basolateral staining of ER in rat distal colon crypts and cytoplasmic staining of intestinal crypts was reported³², indicating a unique subcellular expression pattern of ER in the intestine. Alternative receptor candidates implicated with non-genomic effects of estrogens include the plasma membrane

ER, GPR30 or GPER, and PKC, which are involved in the regulation of distal colonic epithelial Cl^- secretion^{32–34}.

Our findings indicate that basal and acid-stimulated human DBS correlate with endogenous serum estrogen levels. However, the mechanism by which elevation of endogenous estrogen level increases the basal and acid-stimulated DBS in women is not fully understood. Potential explanations include differences in the estrogen release into the lumen, which could explain the observed tonic differences and also the rapid response to perfused estrogen. We have recently found that low concentration of 17β -estradiol (1 nM), which did not stimulate DBS in mice, markedly potentiated prostaglandin E_2 (PGE_2)-stimulated DBS (unpublished data). PGE_2 plays an important role in the regulation of DBS and is the most important intramucosal mediator of acid-stimulated DBS in humans^{3, 4}. Thus, endogenous estrogen may regulate DBS through potentiating the role of endogenous PGE_2 . In addition, 17β -estradiol potentiates membrane currents through cystic fibrosis transmembrane conductance regulator (CFTR) channels³⁵, which can also stimulate DBS.

In summary, we showed that ER are expressed in duodenal mucosa in humans independent of sex and that estrogen stimulates DBS, which may explain the observed lower prevalence of duodenal ulcer in premenopausal women. These new insights may lead to new preventive/therapeutic interventions including the use of estrogenic compounds to protect the gastrointestinal tract in postmenopausal women.

Acknowledgments

Grant Support: This work was supported by National Natural Science Foundation of China (30860092) and International Scientific and Technological Cooperation Program of China (2008DFA32260) to B. Tuo; by an American Heart Association Beginning Grant-in-Aid Award (0565025Y) to H. Dong, by a University of California San Diego Digestive Diseases Research Development Center Grant (DK-080506), in which H. Dong serves as the director of the cell imaging core, and by the National Institutes of Health (HL094728) and the Department of Veterans Affairs to V. Vallon.

Abbreviations used in this paper

CFTR	cystic fibrosis transmembrane conductance regulator
DBS	duodenal bicarbonate secretion
ER	estrogen receptor
<i>H. pylori</i>	<i>Helicobacter pylori</i>
PGE_2	prostaglandin E_2

References

1. Valle, JD.; Chey, WD.; Scheiman, JM. Acid peptic disorders. In: Yamada, T., editor. Textbook of Gastroenterology. 4. Vol. 1. Philadelphia, PA: Lippincott Williams & Wilkins; 2003. p. 1322-1376.
2. Dong WG, Cheng CS, Liu SP, Yu JP. Epidemiology of peptic ulcer disease in Wuhan area of China from 1997 to 2002. World J Gastroenterol. 2004; 10:3377–9. [PubMed: 15484323]
3. Flemstrom G, Isenberg JJ. Gastroduodenal mucosal alkaline secretion and mucosal protection. News Physiol Sci. 2001; 16:23–8. [PubMed: 11390942]
4. Allen A, Flemstrom G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J Physiol Cell Physiol. 2005; 288:C1–19. [PubMed: 15591243]
5. Isenberg JJ, Selling JA, Hogan DL, Koss MA. Impaired proximal duodenal mucosal bicarbonate secretion in patients with duodenal ulcer. N Engl J Med. 1987; 316:374–9. [PubMed: 3807974]
6. Kurata JH, Honda GD, Frankl H. The incidence of duodenal and gastric ulcers in a large health maintenance organization. Am J Public Health. 1985; 75:625–9. [PubMed: 4003625]

7. Rosenstock SJ, Jorgensen T. Prevalence and incidence of peptic ulcer disease in a Danish County--a prospective cohort study. *Gut*. 1995; 36:819–24. [PubMed: 7615266]
8. Vessey MP, Villard-Mackintosh L, Painter R. Oral contraceptives and pregnancy in relation to peptic ulcer. *Contraception*. 1992; 46:349–57. [PubMed: 1486773]
9. Redchits IV, Petrov EE. The age-related characteristics of the clinical picture of duodenal ulcer in women. *Lik Sprava*. 1995:149–52. [PubMed: 8630787]
10. Gerhard M, Ganz P. How do we explain the clinical benefits of estrogen? From bedside to bench. *Circulation*. 1995; 92:5–8. [PubMed: 7788916]
11. Hurn PD, Macrae IM. Estrogen as a neuroprotectant in stroke. *J Cereb Blood Flow Metab*. 2000; 20:631–52. [PubMed: 10779008]
12. Aguwa CN. Effects of exogenous administration of female sex hormones on gastric secretion and ulcer formation in the rat. *Eur J Pharmacol*. 1984; 104:79–84. [PubMed: 6542018]
13. Shimozawa N, Okajima K, Harada N, Arai M, Ishida Y, Shimada S, Kurihara H, Nakagata N. Contribution of sensory neurons to sex difference in the development of stress-induced gastric mucosal injury in mice. *Gastroenterology*. 2006; 131:1826–34. [PubMed: 17087955]
14. Smith A, Contreras C, Ko KH, Chow J, Dong X, Tuo B, Zhang HH, Chen DB, Dong H. Gender-Specific Protection of Estrogen against Gastric Acid-Induced Duodenal Injury: Stimulation of Duodenal Mucosal Bicarbonate Secretion. *Endocrinology*. 2008; 149:4554–4566. [PubMed: 18499763]
15. Yang DT, Owen WE, Ramsay CS, Xie H, Roberts WL. Performance characteristics of eight estradiol immunoassays. *Am J Clin Pathol*. 2004; 122:332–7. [PubMed: 15362362]
16. Thorneycroft IH, Mishell DR Jr, Stone SC, Kharma KM, Nakamura RM. The relation of serum 17-hydroxyprogesterone and estradiol-17-beta levels during the human menstrual cycle. *Am J Obstet Gynecol*. 1971; 111:947–51. [PubMed: 5118032]
17. Maertens C, Wei L, Tytgat J, Droogmans G, Nilius B. Chlorotoxin does not inhibit volume-regulated, calcium-activated and cyclic AMP-activated chloride channels. *Br J Pharmacol*. 2000; 129:791–801. [PubMed: 10683204]
18. Keller SH, Lindstrom J, Taylor P. Inhibition of glucose trimming with castanospermine reduces calnexin association and promotes proteasome degradation of the alpha-subunit of the nicotinic acetylcholine receptor. *J Biol Chem*. 1998; 273:17064–72. [PubMed: 9642271]
19. Oluwole FS, Bolarinwa AF. Effects of gonadectomy on indomethacin-induced ulceration and peptic activity in rats. *Afr J Med Med Sci*. 1990; 19:139–43. [PubMed: 1695811]
20. Rosenstock S, Jorgensen T, Bonnevie O, Andersen L. Risk factors for peptic ulcer disease: a population based prospective cohort study comprising 2416 Danish adults. *Gut*. 2003; 52:186–93. [PubMed: 12524398]
21. Wang JY, Liu SB, Chen SY, Dobson A. Risk factors for peptic ulcer in Shanghai. *Int J Epidemiol*. 1996; 25:638–43. [PubMed: 8671567]
22. Feldman M, Richardson CT, Walsh JH. Sex-related differences in gastrin release and parietal cell sensitivity to gastrin in healthy human beings. *J Clin Invest*. 1983; 71:715–20. [PubMed: 6826731]
23. Isenberg JI, Hogan DL, Selling JA, Koss MA. Duodenal bicarbonate secretion in humans. Role of prostaglandins. *Dig Dis Sci*. 1986; 31:130S. [PubMed: 3080284]
24. Uotinen N, Puustinen R, Pasanen S, Manninen T, Kivineva M, Syvala H, Tuohimaa P, Ylikomi T. Distribution of progesterone receptor in female mouse tissues. *Gen Comp Endocrinol*. 1999; 115:429–41. [PubMed: 10480995]
25. Press MF, Greene GL. Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. *Endocrinology*. 1988; 122:1165–75. [PubMed: 3342750]
26. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, Saladin R, Schoonjans K, Pellicciari R, Auwerx J. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. *J Med Chem*. 2008; 51:1831–41. [PubMed: 18307294]
27. Kawano N, Koji T, Hishikawa Y, Murase K, Murata I, Kohno S. Identification and localization of estrogen receptor alpha- and beta-positive cells in adult male and female mouse intestine at various estrogen levels. *Histochem Cell Biol*. 2004; 121:399–405. [PubMed: 15138841]

28. Taylor AH, Al-Azzawi F. Immunolocalisation of oestrogen receptor beta in human tissues. *J Mol Endocrinol.* 2000; 24:145–55. [PubMed: 10657006]
29. Harvey BJ, Condliffe S, Doolan CM. Sex and salt hormones: rapid effects in epithelia. *News Physiol Sci.* 2001; 16:174–7. [PubMed: 11479368]
30. Li S, Han B, Liu G, Li S, Ouellet J, Labrie F, Pelletier G. Immunocytochemical localization of sex steroid hormone receptors in normal human mammary gland. *J Histochem Cytochem.* 2010; 58:509–15. [PubMed: 20026671]
31. Pelletier G. Localization of androgen and estrogen receptors in rat and primate tissues. *Histol Histopathol.* 2000; 15:1261–70. [PubMed: 11005250]
32. O'Mahony F, Alzamora R, Chung HL, Thomas W, Harvey BJ. Genomic priming of the antisecretory response to estrogen in rat distal colon throughout the estrous cycle. *Mol Endocrinol.* 2009; 23:1885–99. [PubMed: 19846538]
33. O'Mahony F, Alzamora R, Betts V, LaPaix F, Carter D, Irnaten M, Harvey BJ. Female gender-specific inhibition of KCNQ1 channels and chloride secretion by 17beta-estradiol in rat distal colonic crypts. *J Biol Chem.* 2007; 282:24563–73. [PubMed: 17556370]
34. O'Mahony F, Harvey BJ. Sex and estrous cycle-dependent rapid protein kinase signaling actions of estrogen in distal colonic cells. *Steroids.* 2008; 73:889–94. [PubMed: 18374379]
35. Goodstadt L, Powell T, Figtree GA. 17beta-estradiol potentiates the cardiac cystic fibrosis transmembrane conductance regulator chloride current in guinea-pig ventricular myocytes. *J Physiol Sci.* 2006; 56:29–37. [PubMed: 16779911]

Figure 1. Duodenal tube with five channels and three balloons used to functionally isolate human proximal duodenum for measurement of duodenal HCO_3^- secretion. The tube was positioned to the proximal duodenum using a special placing-clips under gastroscopy.

Figure 2.

Epidemiologic study of duodenal ulcer prevalence. (A) Numbers of male and female subjects included in study. There were no significant differences between numbers of men and women in each age group ($P>0.05$). (B) *H. pylori* infection of men and women. There were no significant differences between *H. pylori* infection of men and women in each age group ($P>0.05$). (C) Prevalence of duodenal ulcer in men and women. There were significant differences between duodenal ulcer prevalence of men and women in each age group <70 years, especially in age groups <50 years ($*p<0.05$, $***p<0.001$, and $\#P>0.05$). Male/female ratio of duodenal ulcer prevalence in different age groups is shown at the bottom. (D) Duodenal ulcer prevalences in *H. pylori*-infected and non-*H. pylori*-infected male and female patients younger than 50 years of age ($***P<0.001$).

Figure 3.

Age and sex differences of basal and acid-stimulated DBS in humans. (A and C) Time courses of basal and acid-stimulated DBS in men and women at age of 20–29 years (A) and 60–69 years (C). After measuring basal DBS for 60 min, the duodenum was luminally exposed to 10 ml HCl (100 mM) for 10 min as indicated, and then DBS was measured for additional 60 min. Acid markedly stimulated DBS in all groups (* $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$, comparison with basal value). Maximal responses were reached within 30 min after administration of HCl. (B and D) Basal and acid-stimulated net peak DBS in men and women at age of 20–29 years (B) and 60–69 years (D) (* $P < 0.05$, ** $P < 0.01$, or # $P > 0.05$, comparison between men and women). (E) Basal DBS of men and women at age of 20–29 years and 60–69 years (* $P < 0.05$, or # $P > 0.05$, comparison between two age groups). (F) Acid-stimulated net peak DBS of men and women at age of 20–29 years and 60–69 years (** $P < 0.01$, or # $P > 0.05$, comparison between two age groups).

Figure 4.

Basal and acid-stimulated DBS during the different stages of the menstrual cycle in premenopausal women. (A) Time courses of basal and acid-stimulated DBS during the preovulatory and premenstrual phase in women at the age of 20–29 years. After measuring basal DBS for 60 min, the duodenum was luminally exposed to 10 ml HCl (100 mM) for 10 min as indicated, and then DBS was measured for additional 60 min. Acid markedly stimulated DBS in preovulatory and premenstrual women (* $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$, comparison with basal value). (B) Basal and acid-stimulated net peak DBS during preovulatory and premenstrual phase (* $P < 0.05$, comparison between preovulatory and premenstrual). (C) Serum estradiol levels during preovulatory and premenstrual phase in the same women (*** $P < 0.001$, comparison between preovulatory and premenstrual).

Figure 5.

Effect of 17β -estradiol applied to the duodenal lumen on DBS in men and women at different ages. (A and C) Time courses of basal and 17β -estradiol-stimulated DBS in men and women at the age of 20–29 years (A) and 60–69 years (C). After measuring basal DBS for 60 min, the duodenum was luminally exposed to 10 ml 17β -estradiol (0.1 mg/ml) for 10 min as indicated, and then DBS was measured for additional 75 min (** $P < 0.01$, comparison with basal value). (B and D) Basal and 17β -estradiol-stimulated net peak DBS in men and women at the age of 20–29 years (B) and 60–69 years (D) (* $P < 0.05$, or # $P > 0.05$, comparison between men and women).

Figure 6.

Expression of ER α and ER β proteins in human proximal duodenal mucosa. Duodenal mucosae from healthy men (M) and women (F) at the age of 20–29 years were subjected to Western blot analysis as described in Methods. ER α and ER β were similarly expressed in duodenal mucosae of men and women. Representative blots are shown from 3 separate experiments with identical results.

Figure 7.

Expression of ER α and ER β in duodenal epithelial cells by immunohistochemistry. Human proximal duodenal mucosae (from the same subjects shown in Figure 6) were subjected to immunohistochemistry analysis as described in Methods. Representative pictures are shown from 3 men and 3 women separate experiments with identical results. Both ER α and ER β were mainly localized to plasma membrane and cytosol of villous and crypt cells in duodenal mucosae. (A1 and A2) Expressions of ER α in villous (A1) and crypt (A2) cells. (B1 and B2) Expressions of ER β in villous (B1) and crypt (B2) cells. (C1 and C2) Expressions of ER α (C1) and ER β (C2) in breast carcinoma cells (positive control). (D1 and D2) Expressions of ER α (D1) and ER β (D2) in hepatic carcinoma cells, HepG2 (negative control). All original magnifications for (A) to (D) are x400. (E) Negative control in human proximal duodenal mucosae without primary antibody (original magnification, x100).