

# Histologic Study of the Esophagogastric Junction of Organ Donors Reveals Novel Glandular Structures in Normal Esophageal and Gastric Mucosae

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**INTRODUCTION:** Whether cardiac mucosa at the esophagogastric junction is normal or metaplastic is controversial. Studies attempting to resolve this issue have been limited by the use of superficial pinch biopsies, abnormal esophagi resected typically because of cancer, or autopsy specimens in which tissue autolysis in the stomach obscures histologic findings.

**METHODS:** We performed histologic and immunohistochemical studies of the freshly fixed esophagus and stomach resected from 7 heart-beating, deceased organ donors with no history of esophageal or gastric disease and with minimal or no histologic evidence of esophagitis and gastritis.

**RESULTS:** All subjects had cardiac mucosa, consisting of a mixture of mucous and oxyntic glands with surface foveolar epithelium, at the esophagogastric junction. All also had unique structures we termed compact mucous glands (CMG), which were histologically and immunohistochemically identical to the mucous glands of cardiac mucosa, under esophageal squamous epithelium and, hitherto undescribed, in uninflamed oxyntic mucosa throughout the gastric fundus.

**DISCUSSION:** These findings support cardiac mucosa as a normal anatomic structure and do not support the hypothesis that cardiac mucosa is always metaplastic. However, they do support our novel hypothesis that in the setting of reflux esophagitis, reflux-induced damage to squamous epithelium exposes underlying CMG (which are likely more resistant to acid-peptic damage than squamous epithelium), and proliferation of these CMG as part of a wound-healing process to repair the acid-peptic damage could result in their expansion to the mucosal surface to be recognized as cardiac mucosa of a columnar-lined esophagus.

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## INTRODUCTION

Cardiac mucosa, comprising a surface epithelial compartment of mucus-producing foveolar cells and a deeper epithelial compartment composed of mucous glands or mixed mucous/oxyntic glands, is found frequently at the squamocolumnar junction (SCJ) where the esophagus joins the stomach (Figure 1a) (1). Cardiac mucosa traditionally was assumed to be the normal, congenital lining of the gastric cardia but, in 1997, Chandrasoma proposed that cardiac mucosa is not normal but rather an acquired, gastroesophageal reflux disease (GERD)-induced metaplasia of the esophagus (2). He presented autopsy data to support his hypothesis that squamous mucosa normally joins gastric oxyntic mucosa at the SCJ, but in the setting of chronic reflux esophagitis, cardiac mucosa

develops through a metaplastic process that ultimately can result in the intestinal metaplasia of Barrett's esophagus. In subsequent reports, he proposed that any segment of cardiac mucosa or intestinal metaplasia interposed between esophageal squamous mucosa proximally and gastric oxyntic mucosa distally represents a metaplasia-lined esophageal segment whose length is proportional to the severity of the underlying GERD (3,4).

Histology studies attempting to resolve the issue of whether cardiac mucosa at the SCJ is normal or metaplastic have been limited by the use of: (i) superficial pinch biopsies from patients in endoscopy units who cannot be considered normal subjects (5), (ii) abnormal esophagi resected typically because of cancer (3), or (iii) autopsy specimens in which tissue autolysis in the stomach

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can severely obscure histologic findings at the esophagogastric junction (EGJ) (6). The aim of this prospective study was to evaluate histologic features of the freshly fixed EGJ of organ donors.

## METHODS

Immediately after harvesting the heart and/or lungs from consecutive, heart-beating, deceased organ donors with no history of esophageal or gastric disease, the esophagus and stomach were resected *en bloc* and immediately fixed in formalin. Longitudinally oriented, full-thickness sections were taken across the EGJ (including several centimeters of esophagus proximally and gastric fundus distally) and embedded in paraffin; 5- $\mu$ m serial sections were cut, mounted on slides, and stained with hematoxylin and eosin for routine histologic assessment; immunohistochemical stains for caudal homeobox 2, mucin (MUC)1, MUC2, MUC5, and MUC6 also were performed. Slides were evaluated for type and degree of inflammation in esophagus and stomach; type, location, and extent of mucous glands; presence of goblet cells; and type of mucin expression. The length of the cardiac mucosa segment was measured using a Nikon e400 microscope (Nikon 4 $\times$ /0.10 objective). This study was approved by the Baylor Scott and White Research Institute Institutional Review Board, and by the Southwest Transplant Alliance.

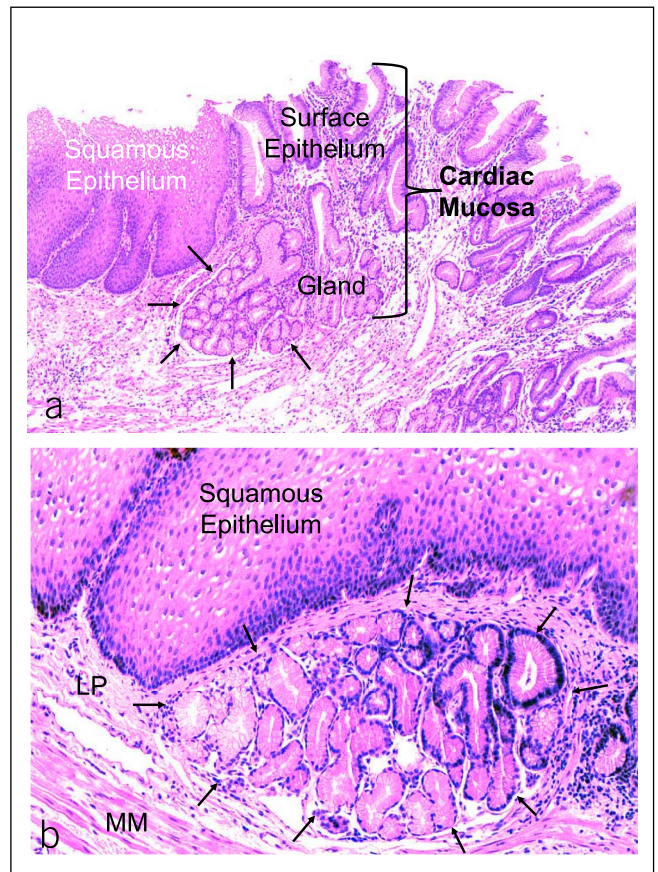
## RESULTS

There were 7 subjects (4 men, 3 women; median age 42, range 20–53 years). One had mild active esophagitis and an acute, stress-type erosion in gastric oxyntic mucosa; the other 6 subjects had no histologic abnormalities of either organ. All 7 subjects had cardiac mucosa averaging 5.7 mm in length (range 1.4–11.0 mm), situated between esophageal squamous mucosa proximally and gastric fundic (oxyntic) mucosa distally (Figure 1a). Inflammation in cardiac mucosa was absent in 4 and mild (lymphocytes only) in 3 subjects who had no other signs of epithelial injury. One subject had goblet cells in cardiac mucosa. Submucosal glands and their ducts were observed underneath squamous mucosa in all cases.

All subjects showed well-lobulated structures we termed compact mucous glands (CMG), similar in appearance to the basal glands of cardiac mucosa and composed of mucus-producing cells indistinguishable from those of cardiac mucosa, in the lamina propria underneath esophageal squamous epithelium at the SCJ (Figure 1b). Surprisingly, all subjects also had multiple CMG throughout the uninflamed gastric fundus; the gastric CMG were located in the lamina propria immediately above the muscularis mucosae (mean 3.3 CMG per 2-cm length of gastric tissue, range 0.0–12.0) (Figures 2a,b). These gastric CMG, hitherto unreported structures, were lined by tall columnar cells with apical mucin droplets identical in histologic appearance and mucin composition (positive for MUC 1, 5, and 6; negative for MUC 2 and caudal homeobox 2) to the subsquamous CMG and the mucus-producing cells of cardiac mucosa at the SCJ. CMG were found in the highest density in the region of fundic (oxyntic) mucosa closest to cardiac mucosa at the EGJ but were present throughout the fundus even in the distal-most segments.

## DISCUSSION

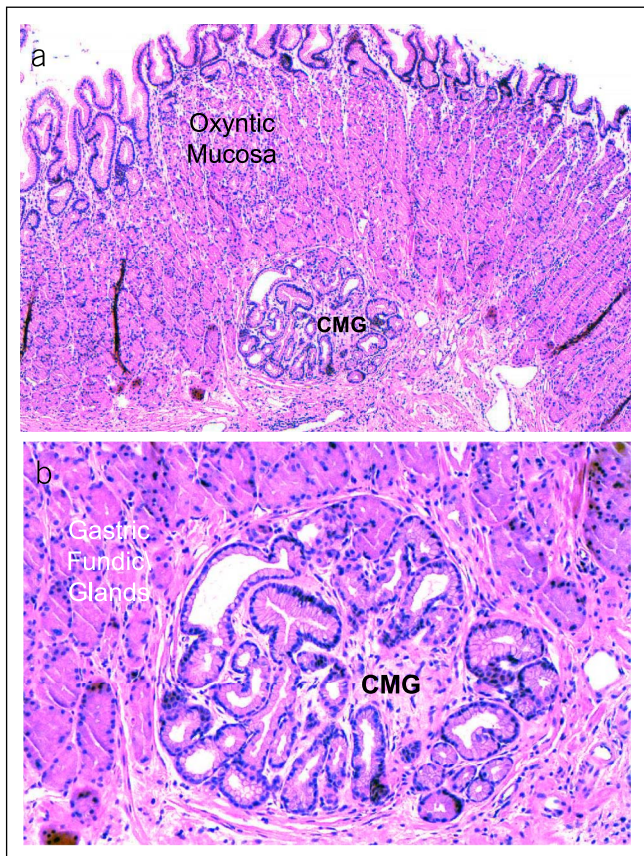
In this histologic study of freshly fixed esophagi from heart-beating, deceased organ donors with no history of esophageal



**Figure 1.** (a) Low-power image of the squamocolumnar junction region of an organ donor subject showing a segment of cardiac mucosa. This mucosa is composed of a surface epithelial compartment of mucus-producing foveolar cells and a deeper epithelial compartment of mucus glands (arrows surround a mucus gland) (hematoxylin and eosin 4 $\times$ ). (b) Tissue specimen from the squamocolumnar junction region showing a subsquamous CMG (surrounded by black arrows) situated in the lamina propria underneath squamous epithelium (hematoxylin and eosin, 10 $\times$ ). CMG are organized, lobular collections of glands lined by mucus cells exclusively, without oxyntic cells or inflammation. CMG are histologically and immunohistochemically identical to the glands of cardiac mucosa. Images were edited in Photoshop for cropping and to enhance clarity. Any adjustments in contrast, color balance, brightness, or sharpness were applied to the entire image. CMG, compact mucus gland; LP, lamina propria; MM, muscularis mucosae.

disease and with minimal or no evidence of esophageal inflammation, we identified a short segment of cardiac mucosa (averaging 5.7 mm in length) at the EGJ in all 7 subjects. This finding argues against the contention that cardiac mucosa always represents a GERD-induced metaplasia (2–4). However, caution is warranted before concluding that a finding is normal simply because it is observed frequently. For example, autopsy studies have found evidence of atherosclerosis in more than 75% of young male trauma victims, but atherosclerosis is not considered a normal condition (7). Furthermore, after esophagectomy with gastric pull-up reconstruction, the observation that cardiac type mucosa often appears in the reflux-damaged esophageal remnant suggests that cardiac type mucosa can develop as an inflammation-induced metaplasia (8). Nevertheless, our finding of a segment of cardiac mucosa in all of our organ donor subjects





**Figure 2.** CMG in oxyntic mucosa of the gastric fundus. (a) Low-power image of a section of the gastric fundus with a CMG located at the base of the gastric oxyntic mucosa (hematoxylin and eosin, 4 $\times$ ). (b) A higher-power image of the same gastric CMG (hematoxylin and eosin, 10 $\times$ ). Gastric CMG are histologically and immunohistochemically similar to the glands of cardiac mucosa shown in Figure 1a and to the subsquamous CMG shown in Figure 1b. Note that there is no inflammation in or surrounding CMG in either squamous or gastric mucosa. Images were edited in Photoshop for cropping and to enhance clarity. Any adjustments in contrast, color balance, brightness, or sharpness were applied to the entire image. CMG, compact mucus gland.

who had little or no evidence of esophageal inflammation does not support the notion that cardiac mucosa is always metaplastic.

All study subjects had uninflamed CMG, composed of organized collections of mucous glands (whose cells are histologically identical to those of cardiac mucosa glands), in the lamina propria underneath squamous epithelium at the SCJ. Perhaps, our most surprising finding was the identification of CMG scattered throughout uninflamed oxyntic mucosa of the gastric fundus in all 7 subjects, with greatest density in the proximal stomach closest to cardiac mucosa. We are unaware of any previous description of CMG in the gastric fundus. Interestingly, the mucus-producing cells of cardiac mucosa, CMG at the SCJ, and CMG in the gastric fundus have identical mucin immunohistochemical staining patterns.

Structures in lamina propria similar to CMG occasionally have been described in the esophagus (9) but not with the frequency that we observed at the SCJ and, to our knowledge, never in the fundus of the uninflamed stomach that is traditionally considered devoid of mucus glands. These gastric CMG are

larger in size but similar in appearance to the glands of pseudopyloric metaplasia (also called spasmodic polypeptide-expressing metaplasia or SPEM), which develops when chronic inflammation and atrophy of oxyntic mucosa in the gastric fundus causes it to resemble the pyloric gland mucosa that normally lines the gastric antrum (10). Indeed, the mucus cells of CMG, cardiac mucosa, and pseudopyloric metaplasia are indistinguishable histologically. We can only speculate as to why CMG in oxyntic mucosa have not been described previously. Because CMG exhibit no evidence of neoplasia or inflammation and are present within normal fundic mucosa, it is likely that they have merely been ignored by surgical pathologists focused on identifying clinically important pathologic abnormalities.

Our finding that CMG seems to be normal structures in subsquamous epithelium of the distal esophagus and throughout the gastric fundus has potentially important implications for the pathogenesis of the columnar-lined esophagus and, perhaps, even pseudopyloric metaplasia. Mucus-secreting CMG in the esophagus are likely to be more resistant to acid-peptic damage than the overlying squamous epithelium. Thus, reflux-induced damage to squamous epithelium of the distal esophagus could expose underlying CMG, whose proliferation as part of a wound-healing process could result in the expansion of mucous cells to the surface where they would be recognized as part of cardiac mucosa. Similarly, in the *Helicobacter pylori*-infected stomach whose parietal cell-containing oxyntic glands are vulnerable to destruction by inflammation, resistant CMG might proliferate and expand to become recognized as pseudopyloric metaplasia. The fact that the mucous cells of cardiac mucosa and pseudopyloric metaplasia are histologically identical is well explained if CMG are their common precursor. Thus, cardiac mucosa and pseudopyloric metaplasia might not develop necessarily through transdifferentiation of mature cells or abnormal differentiation of progenitor cells, as is commonly assumed (10,11), but rather through proliferation of a normal structure that is more resistant to acid-peptic damage than squamous epithelium in the esophagus and to *H. pylori*-induced inflammation than oxyntic glands in the stomach. These novel hypotheses warrant further investigation.

#### CONFLICTS OF INTEREST

**Guarantor of the article:** Stuart J. Spechler, MD.

**Specific author contributions:** Robert Odze and Stuart J. Spechler, MD, contributed equally to this work. R.O.: study concept/design; analysis and interpretation of data; critical revision of manuscript; important intellectual content; and drafting of manuscript. S.J.S.: study concept/design; analysis and interpretation of data; critical revision of manuscript; important intellectual content; and drafting of manuscript. E.P.: study design; technical and material support; important intellectual content; and critical revision of manuscript. A.N.: study design; technical and material support; important intellectual content; and critical revision of manuscript. V.K.: study design; technical and material support; important intellectual content; and critical revision of manuscript. R.F.S.: study concept/design; analysis and interpretation of data; critical revision of manuscript; important intellectual content; and drafting of manuscript.

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**Potential competing interests:** None to report.

## Study Highlights

### WHAT IS KNOWN

- ✓ It is not clear whether cardiac mucosa at the EGJ is normal or metaplastic.
- ✓ Studies that have attempted to resolve this issue have been limited by the use of superficial pinch biopsies, abnormal esophagi resected typically because of cancer, or autopsy specimens in which tissue autolysis in the stomach obscures histologic findings.

### WHAT IS NEW HERE

- ✓ In 7 heart-beating, deceased organ donors with no history of esophageal or gastric disease and with minimal or no histologic evidence of esophagitis and gastritis, we found a short segment of cardiac mucosa (averaging 5.7 mm in length) at the EGJ.
- ✓ All 7 study subjects also had unique structures we termed CMG, which were histologically and immunohistochemically identical to the mucous glands of cardiac mucosa, under esophageal squamous epithelium and, hitherto undescribed, in uninflamed oxyntic mucosa throughout the gastric fundus.

### TRANSLATIONAL IMPACT

- ✓ A short segment of cardiac mucosa at the EGJ appears to be normal and not always a metaplastic structure.
- ✓ We hypothesize that in the setting of reflux esophagitis, reflux-induced damage to squamous epithelium exposes underlying CMG whose proliferation as part of a wound-healing process could result in their expansion to the mucosal surface to be recognized as cardiac mucosa of a columnar-lined esophagus.

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