Manuscript ID: 28458

Title: Sex Differences and Effects of Oestrogen in Rat Gastric Mucosal Defence

Journal: *World Journal of Gastroenterology*

Response to reviewer's comments

Dear editor,

We thank you for your consideration of our manuscript. We appreciate your response and initial feedback, and made modifications to improve the manuscript according to the comments made by the editorial office and the reviewer.

We hope that you will find the revised paper closer to being suitable for publication and we look forward to contributing to your journal. Please do not hesitate to contact us with further questions, comments or concerns regarding the manuscript.

Best regards,

Richard Shore, MD

Corresponding author

Editorial office

1. Please provide the grant application form(s). If you can't provide it, please delete this part.

Response: Grant application forms can't be provided. Part deleted from the manuscript.

2. All files must be signed by the corresponding author and provided in a PDF format.

Response: All files have been signed and are provided in a PDF format.

3. The ethic approval document(s)/letter(s) must be provided in a PDF format, and each statement must also be mentioned as a footnote in the manuscript text.

Response: All procedures involving animals were reviewed and approved by the Regional Ethical Committee for Laboratory Animal Experiments in Uppsala (IACUC protocol (Swedish) number: C288/9). This was added to the manuscript (title page). We provide the approval from our ethical committee.

4. All files must be signed by the corresponding author and provided in a PDF format.

Response: The institutional review board statement reads: "This study was reviewed and approved by the Regional Ethical Committee for Laboratory Animal Experiments in Uppsala" because the institutional review board and the animal care and use committee are the same institutional body in Sweden. The institutional review board statement has been added to the manuscript (title page) and a file signed by the corresponding author has been provided in a PDF format.

5. This file must be provided in a PDF format, and the statement must also be mentioned as a footnote in the manuscript text. ***Sample wording:*** *All study participants, or their legal guardian, provided informed written consent prior to study enrolment.*

Response: This study was conducted in animals. All procedures involving animals were reviewed and approved by the Regional Ethical Committee for Laboratory Animal Experiments in Uppsala. This was added to the manuscript (title page). A file signed by the corresponding author has been provided in a PDF format.

6. This file must be signed by the corresponding author and provided in a PDF format, and the statement must also be mentioned as a footnote in the manuscript text. ***Sample wording:*** *[Name of individual] has received fees for serving as a speaker, a [position; such as consultant and/or an advisory board member] for [name(s) of organization(s)]. [Name of individual] has received research funding from [name(s) of organization(s)]. [Name of individual] is an employee of [name(s) of organization(s)]. [Name of individual] owns stocks and/or shares in [name(s) of organization(s)]. [Name of individual] owns patent [patent identifier information (including patent number, two-letter country code, and kind code) and a brief description].*

Response: There are no conflicts of interest. This was added to the manuscript (title page). A file signed on behalf of all authors by the corresponding author has been provided in a PDF format.

6.x: This was not commented on but listed in the guidelines: A Data sharing statement must be mentioned as a footnote in the manuscript text and this file must be signed by the corresponding author and provided in a PDF format.

Response: No additional data are available. This was added to the manuscript (title page). A file signed by the corresponding author has been provided in a PDF format.

7. This file must be signed by the corresponding author and provided in a PDF format, and the statement must also be mentioned in the text. ***Example wording:*** *The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23°C, 12h/12h light/dark, 50% humidity, ad libitum access to food and water) for two weeks prior to experimentation. Intragastric gavage administration was*

carried out with conscious animals, using straight gavage needles appropriate for the animal size (15-17 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). All animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) for tissue collection.

Response: The animal protocol was designed to minimise pain or discomfort to the animals. The rats were kept in standardised conditions of temperature (21-22°C) and ambient lighting using a 12 hour night and day cycle. Rats were allowed to acclimatise in wide cages with a mesh bottom and free access to pelleted food and tap water for at least seven days before the experiments started. Before each experiment they were fasted for 18-20 hours with free access to water. To avoid additional stress the animals were handled as little as possible outside their cages and administered 120 mg kg^{-1} of thiobutabarbital sodium intraperitoneally to induce anaesthesia before being returned to the cage. After an appropriate length of time the level of anaesthesia was evaluated by the reactions of the eye-lid and foot to tactile and painful stimulus respectively. If anaesthesia was deemed non-sufficient an additional bolus of thiobutabarbital sodium was administered when necessary during the experimental procedure. No opioids or other analgesics were administered to the animals. Core body temperature was kept at 37-38°C using a heating pad connected to a rectal thermistor. A PE-200 cannula was inserted into the trachea to facilitate spontaneous breathing. After the end of each experiment all animals were euthanised with an intravenous injection of saturated potassium chloride solution.

This animal care and use statement has been added to the manuscript (title page) and a file signed by the corresponding author has been provided in a PDF format.

8. This statement must be mentioned in the text, and a certificate of statistical review signed by a biostatistician must be provided in PDF format. ***Sample wording:*** *The statistical methods of this study were reviewed by [name(s) of individual(s)] from [name(s) of organization(s)]…*

Response: The statistical methods of this study were reviewed and approved by Biostatistician Huan Song of the Karolinska Institutet. This was added to the manuscript (title page). A file signed by both the Biostatistician and by the corresponding author has been provided in a PDF format.

9. Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed)

*DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.**)*

For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.

Response: The references have been revised throughout to include PMID and DOI. In references where a DOI could not be found using the free DOI name lookup tool Crossref (http://www.crossref.org/guestquery/) or a page/tool which uses Crossref (http://watcut.uwaterloo.ca/tools/pmid2doi) the statement "No DOI found" was inserted.

10. Please provide the decomposable figure of Figures, whose parts are movable and can be edited. So please put the original picture as word or ppt or excel format so that I can edit them easily.

Response: When converted from .tif (the picture format produced by GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego, CA, USA, which was also used for statistical calculations) to word, ppt or excel formats figures 1 through 5 only turn out as pictures where no changes can be made. Therefore, if you require any form of editing to be done to these pictures please get back to us and we will manually edit any or all of the .tif originals accordingly.

Reviewer #1

1. In the introduction and conclusions the authors made extensive references to gastric cancer and hypothesized that a potentially protective effect of estrogen could be exerted by influencing the mechanisms of gastric mucosal defense including blood flow. Since they did not study gastric cancer model in rat (e.g. nitrosoguanidine-induced gastric cancer in rats) nor the effect of estrogens in this model they could not test their hypothesis. Cancer involves/requires epithelial metaplasia, dysplasia, reduced tumor suppressor genes, immune system involvement, stem cells etc. Therefore, in this paper the authors should stick to their findings, which are interesting.

Response: We agree with the Reviewer. The Introduction, Discussion, Conclusions and Comments have been edited to this effect. The scope has instead been broadened to encompass gastric diseases such as peptic ulcer disease and gastro-duodenal injury.

2. While blood flow is one of the important mechanisms of mucosal defense, as is mucus there are other factors that are important and should be at least mentioned and possibly studied. References on gastric mucosal defense should be updated, e.g. they should cite - Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology 135:41-60, 2008 and newer papers published in the WJG.

Response: We agree with the Reviewer. The introduction has been updated with other factors that are important mechanisms of mucosal defence. Following these additions, references have been updated and extended.

3. In the manuscript the authors stated that "CGRP staining was present in the cytosol of gastric glands, infiltrating cells, myenteric neurons of the muscularis and in endothelial cells of blood vessels. CGRP staining was less than for ERa and ERβ and was only observed in the cytosol of endothelial cells." The authors should elaborate on the distribution of above receptors in endothelial cells of mucosal microvessels provide extensive, good quality illustration (pictures) for ERa, ERβ and CGRP staining.

Response: We thank the reviewer for this comment. Our intensions when analysing the immunohistochemistry was not to specify the exact intracellular locations of the different receptors but to make rough overall comparisons of receptor staining and gross cellular distributions between the sexes. The intensity of staining in our samples was week (see last paragraph of section E under methods) and only allows for observations of receptor staining. It is not sufficient to draw firm conclusions about the precise distributions of these receptors. We consider any statements on intracellular locations to be irrelevant to our overall goal and have not made any effort to confirm these findings. As such, any previous references to intracellular receptor distribution have been omitted from the manuscript. Moreover, figure 6 has been completely overhauled to provide extensive, good quality pictures of ERα, ERβ and CGRP staining.

4. Language evaluation: Grade B: minor language polishing

Response: The corresponding author is bilingual, raised by an English father and a Swedish mother. However, the revised manuscript has been submitted for professional proofreading and will be re-submitted to the journal proofread upon request.