

# Subjective food hypersensitivity: assessment of enterochromaffin cell markers in blood and gut lavage fluid

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**Background:** Food hypersensitivity is commonly suspected, but seldom verified. Patients with subjective food hypersensitivity suffer from both intestinal and extraintestinal health complaints. Abnormalities of the enterochromaffin cells may play a role in the pathogenesis. The aim of this study was to investigate enterochromaffin cell function in patients with subjective food hypersensitivity by measuring serum chromogranin A (CgA) and 5-hydroxytryptamine (5-HT, serotonin) in gut lavage fluid.

**Methods:** Sixty-nine patients with subjective food hypersensitivity were examined. Twenty-three patients with inflammatory bowel disease and 35 healthy volunteers were included as comparison groups. CgA was measured in serum by enzyme-linked immunosorbent assay. Gut lavage fluid was obtained by administering 2 L of polyethylene glycol solution intraduodenally. The first clear fluid passed per rectum was collected and 5-HT was analyzed by liquid chromatography tandem mass spectrometry.

**Results:** Serum levels of CgA were significantly lower in patients with subjective food hypersensitivity than in healthy controls ( $P = 0.04$ ). No differences were found in 5-HT levels in gut lavage fluid between patients with subjective food hypersensitivity and the control groups. There was no correlation between serum CgA and gut lavage 5-HT.

**Conclusion:** Decreased blood levels of CgA suggest neuroendocrine alterations in patients with subjective food hypersensitivity. However, 5-HT levels in gut lavage fluid were normal.

**Keywords:** food hypersensitivity, chromogranin A, serotonin, gut lavage fluid, liquid chromatography

## Introduction

Food hypersensitivity is commonly reported, but often remains unexplained, despite extensive medical examinations. Patients with such subjective food hypersensitivity attribute a number of somatic health complaints to the ingestion of specific foods, most often cow's milk, wheat products, egg, fruits, and vegetables.<sup>2</sup> The abdominal symptoms are typically consistent with irritable bowel syndrome.<sup>2</sup> Psychological disturbances are often associated,<sup>3</sup> but do not seem to be major predictors of either gastrointestinal or extraintestinal symptom severity.<sup>4</sup> Hence, the etiology of subjective food hypersensitivity remains obscure.

Alterations of the gastrointestinal enterochromaffin cell population has been described in patients with functional gastrointestinal disorders,<sup>5,6</sup> and are conceivably implicated in the pathogenesis of subjective food hypersensitivity. In a preliminary study, we recently observed low serum levels of chromogranin A (CgA) in patients with subjective food hypersensitivity.<sup>7</sup> CgA is stored and secreted together with amines

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and peptides from the diffuse neuroendocrine system, and serve as a precursor for several peptides with regulatory functions.<sup>8</sup> Enterochromaffin cells are a major source for circulating CgA. In addition, enterochromaffin cells secrete 5-hydroxytryptamine (5-HT, serotonin), a signaling molecule regulating several gut functions, including secretion, motility, and sensitivity. Indeed, most of the body's 5-HT is contained within enterochromaffin cells. High systemic levels of 5-HT may cause symptoms resembling hypersensitivity reactions to food, such as diarrhea,<sup>9,10</sup> nausea,<sup>11</sup> or flushing and heart palpitations.<sup>12</sup>

5-HT is commonly measured in platelets or in plasma, the latter being a poor matrix due to rapid degradation of 5-HT into 5-hydroxyindolacetic acid. More than 95% of plasma 5-HT is present in platelets, and studies assessing platelet 5-HT content in patients with irritable bowel syndrome are somewhat conflicting.<sup>13–15</sup> Alterations of 5-HT secretion within the gut are not necessarily reflected in the systemic circulation, and assessing 5-HT in gut tissue samples is often considered to be more accurate. However, the surface of the gastrointestinal tract is very large, and biopsies can only give information from a small fragment of this huge area. Indeed, enterochromaffin cell counts seem to vary a lot in different studies.<sup>16,17</sup> Our group has developed a method for analyzing “whole gut 5-HT”, by analyzing gut lavage fluid.<sup>18</sup> Enterochromaffin cells release 5-HT mainly from granules at the basal border, but studies have shown that enterochromaffin cells are bipolar and are able to secrete 5-HT both basally, into the systemic circulation, and apically, into the gut lumen.<sup>19–21</sup> Our method quantifies luminal enterochromaffin cell secretion along the entire length of the small and large bowel.

The aim of the present study was to explore whether patients with subjective food hypersensitivity display abnormal enterochromaffin cell functions. To evaluate this, we extended our preliminary observations<sup>7</sup> and employed our recently developed liquid chromatography tandem mass spectrometry (LCMS) method for determination of 5-HT in gut lavage fluid.<sup>18</sup>

## Materials and methods

### Patients

This study included 69 consecutive patients with subjective food hypersensitivity and abdominal complaints (55 females and 14 males, mean age 39 years, range 21–83 years). The patients went through an extensive medical examination, including detailed medical history-taking, physical examination, and routine laboratory tests, in addition to skin-prick

tests, measurements of total and food-specific IgE levels in serum, and double-blind, placebo-controlled food challenge. Gastroenterological investigations included a gastroscopy with gastric and duodenal biopsy-taking to exclude *Helicobacter pylori* infection and celiac disease. Intestinal permeability was assessed as previously described,<sup>22</sup> and levels of calprotectin in gut lavage fluid samples were analyzed to exclude inflammatory bowel disease.<sup>23</sup> Patients with organic gastrointestinal diseases, IgE-mediated food allergies, and pregnant or lactating women were not included. A screening questionnaire based on the Rome II criteria<sup>24</sup> was applied in all patients for the diagnosis of irritable bowel syndrome.

### Control groups

Twenty-seven patients admitted to the Section of Gastroenterology at Haukeland University Hospital because of suspected inflammatory bowel disease were included as a “patient control group” (11 females and 16 males, mean age 35 years, range 21–65 years). A control group consisting of 35 healthy volunteers (24 females and 11 males, mean age 33 years, range 23–61 years) was also included. The participants in this group were mainly employees of National Institute of Nutrition and Seafood Research or Haukeland University Hospital and students at the University of Bergen. Pregnant or lactating women were not included. The study was performed in accordance with the Declaration of Helsinki and was approved by the Regional Committee for Medical Research Ethics.

### Intestinal lavage

The procedure was performed in the morning and all the participants were fasting from midnight. The method of intestinal lavage has been described previously.<sup>22</sup> Briefly, 2 L of isotonic polyethylene glycol solution (MW 3350, Laxabon®, Tika, Sweden) was administered through a nasoduodenal feeding tube over a period of 40 minutes, using a peristaltic pump (505S/RL; Watson Marlow, Falmouth, UK). Approximately 50 µCi of <sup>51</sup>Cr-labeled ethylenediaminetetra-acetic acid (<sup>51</sup>CrEDTA, Amersham, Little Chalfont, UK), was added to the solution to allow estimation of intestinal permeability.<sup>22</sup> A slightly increased intestinal permeability has been reported previously in patients with subjective food hypersensitivity<sup>25</sup> and this parameter was not included as a part of the present report. About one hour after the start of the polyethylene glycol infusion, the solution reached the distal colon and bowel movements started. The first clear fluid passed per rectum was collected and filtered through gauze, and a 4 mL aliquot was collected on tubes containing 0.5 mL of a solution with antiseptic and

antiproteolytic activity prepared by adding 1 mL of 10% sodium azide ( $\text{NaN}_3$ ) to 50 mL of soybean trypsin inhibitor (Sigma, Taufkirchen, Germany). The samples were stored at  $-80^\circ\text{C}$  until analysis.

## Serotonin analysis

5-HT was analyzed by LCMS as described previously.<sup>18</sup> Briefly, a sample of gut lavage fluid was thawed at room temperature and centrifuged. The supernatant was collected and filtered using a hydrophilic nylon membrane syringe filter, 4 mm diameter and 0.45  $\mu\text{m}$  pore size (Chromacol Ltd, Trumbull, CT). Aliquots of 50  $\mu\text{L}$  of the filtered supernatants were transferred into tubes containing internal standard, which was evaporated to dryness in advance. The tubes were vortex-mixed for 1 minute, transferred to an autosampler vial and submitted to LCMS/MS analysis. The internal standard 5-methoxytryptamine (5- $\text{CH}_3\text{O-HT}$ ) was purchased from Sigma.

The LCMS system used in this study was an Agilent 1100 series LC/MSD trap, SL model with an electrospray interface, a quaternary pump, degasser, autosampler, thermostated column compartment, variable wavelength ultraviolet light detector, and 25  $\mu\text{L}$  injection volume. The column was a Zorbax Eclipse- $\text{C}_8$  RP 150  $\times$  4.6 mm, 5  $\mu\text{m}$  (Agilent Technologies, Palo Alto, CA). The solvent system operated in gradient mode at 0.2 mL/min and consisted of water with formic acid 0.1% v/v and acetonitrile, and ultraviolet detection at 254 nm. Complete system control, data acquisition and processing were done using the ChemStation for LC/MSD version 4.2 from Agilent. The transitions monitored were 177  $\rightarrow$  160  $m/z$  for 5-HT and 191  $\rightarrow$  174  $m/z$  for 5- $\text{CH}_3\text{O-HT}$ .

## Chromogranin A analysis

Fasting venous blood samples were collected at 8.30 am using gel vials with no added anticoagulants. The patients were fasting for 10 hours. Serum CgA was measured by enzyme-linked immunosorbent assay following the manufacturer's instructions (ALPCO Diagnostics, Salem, NH).

## Statistics

Data were analyzed and displayed using the GraphPad Prism statistical software package (v 5.00 for Windows; GraphPad Software, San Diego, CA). Log-transformed serotonin levels were evaluated by analysis of variance, whereas CgA levels were evaluated by using unpaired  $t$ -tests. All tests were two-tailed;  $P$  values of less than 5% were considered statistically significant.

## Results

### Subjects

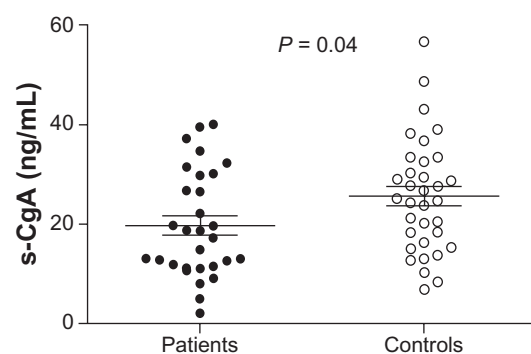
Organic gastrointestinal diseases were not demonstrated in any of the patients with subjective food hypersensitivity. Sixty-four (95%) of the 69 included patients with subjective food hypersensitivity had irritable bowel syndrome according to Rome II criteria, and all completed the lavage procedure. Blood samples for CgA analysis were obtained from 32 of the patients. All of the 27 control patients with suspected inflammatory bowel disease completed the lavage procedure. However, blood samples were not obtained from this group. Based on the medical examination, including endoscopy and histological assessment of mucosal biopsies, 23 of 27 was diagnosed with inflammatory bowel disease, 19 had Crohn's disease, and four had ulcerative colitis. The remaining patients ( $n = 4$ ) were excluded from the study. Thirty-five healthy volunteers were included, and blood samples for CgA analysis were obtained from all of them. Twenty of the 35 controls agreed to participate in the lavage procedure.

### Chromogranin analysis

Serum CgA samples from two of the 32 patients with subjective food hypersensitivity and one of the 35 healthy controls were excluded due to use of proton pump inhibitors, which elevate CgA in blood. Serum levels of CgA were significantly lower in patients with subjective food hypersensitivity compared with healthy controls ( $P = 0.04$ , Figure 1).

### Serotonin analysis

No significant differences in 5-HT levels were demonstrated between patients with subjective food hypersensitivity and the control groups. No differences in 5-HT levels were found between the two control groups either. In the patient group and the healthy volunteer group, two of 69 samples and



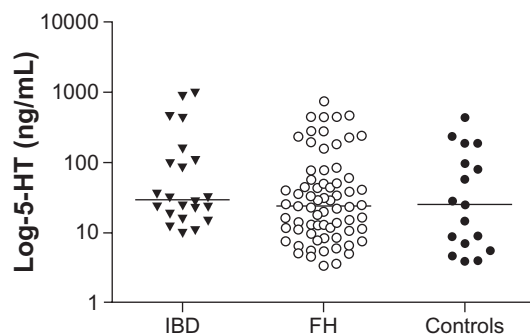
**Figure 1** Serum chromogranin A levels in patients with subjective food hypersensitivity ( $n = 30$ ) and healthy controls ( $n = 34$ ).

**Note:** Individual values with means  $\pm$  standard error are displayed and the  $P$  value is indicated.

three of 20 samples, respectively, were nondetectable. The 5-HT analysis revealed a high degree of variance within all three groups. There was a trend towards a higher level of 5-HT in the inflammatory bowel disease group, albeit not statistically significant (Figure 2). There was no correlation between serum CgA levels and whole gut lavage 5-HT levels, neither in patients ( $r = 0.00005$ , not statistically significant) nor in controls ( $r = 0.08$ ,  $P =$  not statistically significant, Figure 3).

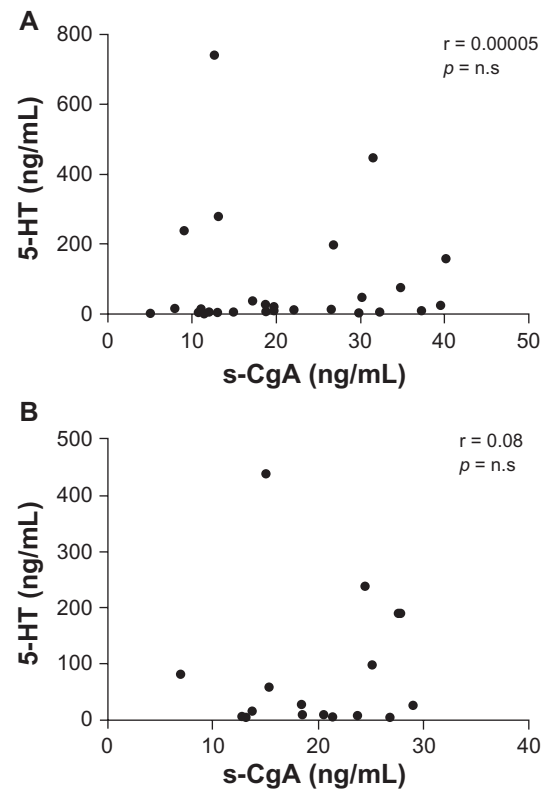
## Discussion

Most intestinal enterochromaffin cells are of the “open” type with apical cytoplasmic extensions which project into the gut lumen and short microvilli,<sup>26</sup> where they sense and respond to the luminal contents by releasing regulatory compounds like 5-HT and CgA, both into the circulation and in the intestinal lumen. Hyperplasia of enterochromaffin cells together with an increase in 5-HT containing granules has been reported in several studies of both functional disorders and inflammatory diseases of the gut,<sup>14,27,28</sup> and 5-HT is supposed to be involved in the pathogenesis of various gastrointestinal disorders.<sup>29–31</sup> However, in the present study, gut lavage 5-HT were found to be normal in patients with subjective food hypersensitivity. Patients with inflammatory bowel disease had slightly higher mean levels of 5-HT, but the difference was not significant. The high degree of variance in the 5-HT data may be due to high individual differences, as well as a consequence of the preanalytical sampling procedure. The use of gut lavage fluid for analytical purposes is unique. Based on our previous experience with the procedure,<sup>22,23</sup> the first clear fluid was chosen because it was desirable to have as few particles as possible to avoid analytical problems, but we acknowledge that the time point of sampling was not necessarily the same for each participant.



**Figure 2** Log-transformed serotonin levels in gut lavage fluid in patients with inflammatory bowel disease ( $n = 23$ ), subjective food hypersensitivity ( $n = 67$ ) and controls ( $n = 17$ ).

**Note:** Individual values with median are displayed. No significant differences between the three groups were observed.



**Figure 3** Relationship between serum chromogranin A levels and gut lavage 5-hydroxytryptamine (5-HT) levels in (A) patients ( $n = 28$ ) with subjective food hypersensitivity and in (B) healthy controls ( $n = 17$ ).

**Note:** Pearson's  $r$  and  $P$  values are indicated.

Wang et al demonstrated an interesting immunoendocrine axis in the gut, where 5-HT production in enterochromaffin cells increases in response to secretory products from CD4<sup>+</sup> T cells.<sup>32</sup> Production and secretion of 5-HT from enterochromaffin cells may thus depend on the immunological profile of the immune response,<sup>33</sup> and there is also evidence that mucosal 5-HT modulates the immune response via serotonergic receptors expressed by immune cells.<sup>34</sup> Indeed, identification of multiple 5-HT receptor subtypes in the gut, especially 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors, suggests multifaceted actions of 5-HT, and have led to the development of several therapeutic agents for functional gastrointestinal disorders.<sup>35</sup>

Serum CgA was significantly lower in the patients with subjective food hypersensitivity compared with the healthy controls, which is consistent with our previous results.<sup>7</sup> Sidhu et al have reported abnormally elevated levels of serum CgA in a small proportion of their patients with diarrhea-predominant irritable bowel syndrome.<sup>36</sup> However, they included no healthy controls, and in many of the patients with elevated CgA, the levels declined with time, which the authors explain as short-lived enterochromaffin cell hyperplasia, as previously reported by Dunlop et al.<sup>6</sup> Thus, it would

be of interest to perform serial measurements of CgA during a day, and also for several continuous days, in future studies to verify possible fluctuations in circulating CgA levels.

Although CgA is a major enteroendocrine secretion product, little is known about its potential role in gastrointestinal pathophysiology. CgA serves as a prohormone for shorter peptide fragments with regulatory properties.<sup>8</sup> Fragments of CgA exert antimicrobial effects and may modulate gastrointestinal motility, sensitivity, and barrier function.<sup>37,38</sup> The low levels of CgA demonstrated in the present study could be a consequence of alterations in the gut microbial flora in patients with subjective food hypersensitivity.<sup>39,40</sup> Intriguingly, Dlugoz et al have recently demonstrated *Chlamydia trachomatis* antigens in enteroendocrine cells and macrophages of the small bowel in patients with irritable bowel syndrome.<sup>41</sup> Although the clinical significance is yet unknown, this is an exciting finding that, together with the present finding of low serum levels of CgA, could imply impairment of enterochromaffin cell function in patients with subjective food hypersensitivity and irritable bowel syndrome. El-Salhy et al recently demonstrated decreased CgA-positive cells in biopsies from patients with irritable bowel syndrome.<sup>42</sup> Thus, a decrease in numbers of CgA secretory cells could be an explanation for the low serum CgA found in the present study.

## Conclusion

We conclude that measurements of systemic CgA reveal significantly lower levels in patients with subjective food hypersensitivity than in healthy controls, whereas “whole gut 5-HT” levels seem to be normal. Impaired secretion of CgA may play a role in functional gastrointestinal disorders, and warrants further investigation.

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## Disclosure

The authors report no conflicts of interest in this work.

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