Introduction

Dietary habits are among the external factors that exert the most influence on the composition of the intestinal microbiota (1–3). To date, several studies have evaluated the impact of different macro and micronutrients on intestinal microbial patterns (4–6). It has been demonstrated that a high consumption of animal proteins, saturated fat, sugar, and salt could weaken the intestinal barrier due to a higher growth of certain pathogenic bacteria (1). On the contrary, a diet rich in complex polysaccharides and plant protein could be related to an increase of certain health-related bacteria capable of stimulating the production of short-chain fatty acids (SCFA) in the intestines (1).

Several disorders affecting the gastrointestinal tract, such as food intolerances, irritable bowel syndrome (IBS) or celiac disease, can be managed by following highly restrictive diets (7–11). However, over time, these diets can have a negative impact on the composition of the intestinal microbiota, although this is a more complex relationship (11–15). In a recent study by Lenhart et al. IBS patients following low-FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols), gluten-free or dairy-free diets showed significant differences in bacterial beta diversity and a reduction in the abundance of *Bifidobacterium*, *Lactobacillus,* and *Prevotella* genera (13). Similarly, De Palma et al. reported a reduction in the genera *Bifidobacterium* and *Lactobacillus* in healthy individuals following a gluten-free diet (14). These unwanted effects could be explained by the exclusion of FODMAPs and lactose, described as dietary carbohydrates with prebiotic actions, and gluten-containing foods such as wheat or barley, which are a source of prebiotic fructans (15–17).

Histamine intolerance is a food-related disorder caused by impaired histamine degradation at the intestinal level due to a deficiency in the enzyme diamine oxidase (DAO) (10). Affected individuals suffer a wide range of gastrointestinal and extraintestinal symptoms, such as bloating, diarrhea, abdominal pain, postprandial fullness, constipation, flatulences, headache, tachycardia, hypotonia, pruritus, eczema, urticaria, rhinitis, and nasal congestion. These manifestations usually appear after the consumption of foods containing histamine and/or other biogenic amines (18). In the last decade, new evidence regarding the aetiopathogenesis of histamine intolerance has been published (19–23). Several single nucleotide polymorphisms encoding a DAO enzyme with reduced histamine degradation capacity have been described as potential genetic causes of histamine intolerance (19, 23). Moreover, impaired DAO activity can also be temporary and reversible, being secondary to certain gastrointestinal disorders or as a side effect of some widely used pharmacological drugs (20). More recently, two studies have suggested that dysbiosis of the intestinal microbiota may play a role in this condition (24, 25). In 2018, Schink et al. demonstrated that patients with symptoms of histamine intolerance have an imbalance of the gut microbiota and an impaired intestinal barrier, which could lead to a deficiency in DAO catabolic activity (24). According to these authors, intestinal dysbiosis may contribute to mucosal inflammation and, in turn, favor the development of a leaky gut with the subsequent potential reduction of DAO enzymatic activity. Intestinal inflammation affecting mucosal integrity have been identified as a cause of low DAO activity by other previous works (26–28). In addition, it may be hypothesized that intestinal dysbiosis could be related to histamine intolerance by a possible higher presence of histaminogenic bacteria that could favor the accumulation of histamine at intestinal level, as well as, by a lower presence of bacteria with histaminolytic activity. In fact, the study performed by Mou et al. identified a total of 117 species from the human gut microbiome with the ability to form histamine (29). Moreover, Sánchez-Pérez et al. recently reported a higher proportion of histamine-secreting bacteria (e.g., *Staphylococcus*, *Proteus*, *Clostridium perfringens,* and *Enterococcus faecalis*) in patients with histamine intolerance in comparison with a healthy control group. These authors also reported alterations in gut bacterial diversity in histamine intolerant individuals (25). It would be important to consider that an intestinal dysbiosis could not be, by itself, the only cause of histamine intolerance but would probably aggravate the symptoms derived from other primary causes of a DAO deficiency (genetic or pathological). In fact, a dysbiosis could help explain the varying severity of symptoms frequently reported in individuals with histamine intolerance (30).

The usual dietary management of histamine intolerance is the follow-up of a low-histamine diet. These diets are based on the exclusion of histamine-containing foods (10, 31). As this amine in foods is mainly formed by the bacterial decarboxylation of the precursor aminoacid histidine, the foods susceptible to contain high histamine levels are those fermented or microbiologically altered (by the action of the fermentative bacteria or spoilage bacteria, respectively) (32, 33). Thus, dry-fermented sausages, cured cheese, and other fermented products, together with preserved and semi-preserved fish derivatives, can easily accumulate high histamine levels. Moreover, certain other foods, such as citrus fruits, strawberry, banana and nuts, that do not contain histamine but may contain other biogenic amines (e.g., putrescine and/or cadaverine) are also frequently excluded within low-histamine diets (31). The presence of these other amines can exert an inhibitory effect on the degradation of histamine by DAO enzyme due to the competition for this degradation system (18). Finally, the dietary treatment of histamine intolerance also considers the supplementation with exogenous DAO enzyme to enhance the intestinal degradation of histamine (10, 31).

There is still a lack of information about what happens to the gut microbiota when histamine intolerant patients are treated with a restrictive low-histamine diet and DAO supplementation. Therefore, a first preliminary study was carried out with the

aim to evaluate potential changes on the composition of the intestinal microbiota in a group of five women undergoing the dietary treatment of histamine intolerance.

Materials and methods

Study design

A previous study was conducted in histamine intolerant patients and healthy individuals to characterize and compare the intestinal microbiota composition (25). The current pilot study was carried out with a subgroup of five women from the histamine intolerant group who presented at least three symptoms associated with histamine intolerance. The inclusion criteria were as follows: age between 18 and 65 years; diagnosis of histamine intolerance based on two or more symptoms and negative results for food allergen-specific IgE. The exclusion criteria were pregnancy, lactation, having started a low-histamine diet, and having taken antibiotics and/or probiotics the month before the study. Considering that the female sex seems prevalently affected by histamine intolerance (34), five women were included in the present pilot study. These women were patients from a nutrition and dietetic center specialized in the dietary management of histamine intolerance (DAO Deficiency Clinical Institute, Barcelona, Spain), where the follow-up of the dietary treatment, as well as the evolution of the symptomatology, were monitored. **Table 1** displays the baseline characteristics of each patient (age, serum DAO activity, and symptoms). All women showed deficiency in serum DAO activity. The reported clinical manifestations were mainly gastrointestinal (i.e., bloating, heart burn, flatulence, diarrhea, and abdominal pain), followed by headache and dermatological complaints, such as pruritus and eczema. One patient also reported insomnia, muscular pain, and articular pain.

The current study aimed to evaluate the microbiota composition of these patients along 9-month of this dietary treatment (low-histamine diet and DAO supplementation). **Figure 1** summarizes the study protocol, in which a total of four stool samples of each patient were collected: at baseline (before starting the dietary treatment) and at 2, 6, and 9 months of the study. Moreover, **Figure 1** also details the phases of the dietary treatment prescribed by the nutrition and dietetic center specialized in the dietary management of histamine inolerance. The first phase was the shortest but also the most restrictive, involving the exclusion of all foods with histamine, and/or other biogenic amines. In the second phase, some excluded foods, mainly those without or with low histamine levels but containing other biogenic amines, were gradually reintroduced. Finally, in the last phase, the rest of excluded foods were progressively reintegrated as much as possible according to interindividual tolerance to histamine content in order to achieve a properly long-term balanced diet. During all phases, DAO supplements formulated with porcine kidney protein extract were administered 20 min prior to each main meal (**Figure 1**).

TABLE 1 Baseline characteristics of each patient with histamine intolerance.

Patient	Age (years)	DAO activity (U/mL)	Clinical symptoms
1	65	9.55	Headache, abdominal bloating, and heart burn.
2	31	6.50	Diarrhea, flatulences, and abdominal bloating.
3	44	9.80	Headache, diarrhea, abdominal bloating, articular pain, muscular pain, and insomnia
4	27	7.70	Diarrhea, abdominal pain, flatulences, and abdominal bloating.
5	56	9.15	Headache, eczema, and pruritus.

Plasma DAO activity was also analyzed using a Radio Extraction Assay (REA) according to the manufacturer's instructions (Sciotec Diagnostic Technologies, Tulln, Austria). Values below 10 U/mL (Histamine Degrading Units) are considered as DAO deficient.

All participants were given detailed information about the aim and procedure of the study and gave their written informed consent prior to study inclusion. The study was approved by the Bioethics Committee of the University of Barcelona (IRB00003099).

Determinations in stool samples

The composition of the gut microbiota was determined by isolation of the bacterial DNA from stool samples (QIAamp Power Fecal Pro DNA kit, QIAGEN, Germantown, MD, USA) and subsequent sequencing of the V3-V4 region of 16S rRNA (Illumina MiSeq platform) at the Genomic and Bioinformatic Service of the Autonomous University of Barcelona. Bioinformatics analysis of the microbiota composition was performed with the EzBiocloud Database (ChunLab, Inc., Seoul, Korea). For the 16S rRNA amplicons, baseline sequence data were previously deposited in the NCBI database by the Bioproject PRJNA811749 (25). The sequence data related to months 2, 6, and 9 of the dietary treatment were deposited in the NCBI database by the Bioproject PRJNA842201.

Statistical analysis

Differences in the microbiota composition during the 9-month study were analyzed by the Kruskal–Wallis test for

non-parametric data. Alpha diversity was measured using the Shannon index and Simpson's index, and beta diversity was calculated by Bray–Curtis dissimilarity analysis and visualized using principal coordinates analysis (PCoA). *p*-values of $p < 0.05$ were considered statistically significant.

Results and discussion

The follow-up of the improvement of the symptoms derived from the dietary treatment of each patient was carried out by the nutritionist of the dietetic center specialized in the dietary management of histamine intolerance. According to this information, all patients experienced a general improvement in the baseline symptoms. Gastrointestinal manifestations (i.e., bloating, diarrhea, flatulences, abdominal pain, and heart burn) were those that mainly disappeared during the dietary treatment most of them already from the second month. Headache improved in two out of the three patients that reported this symptom. Despite these improvements, any patient achieved a total remission of clinical manifestations.

Regarding the composition of the intestinal microbiota, a gut dysbiosis was evidenced in all the recruited women when compared with the results obtained for a group of

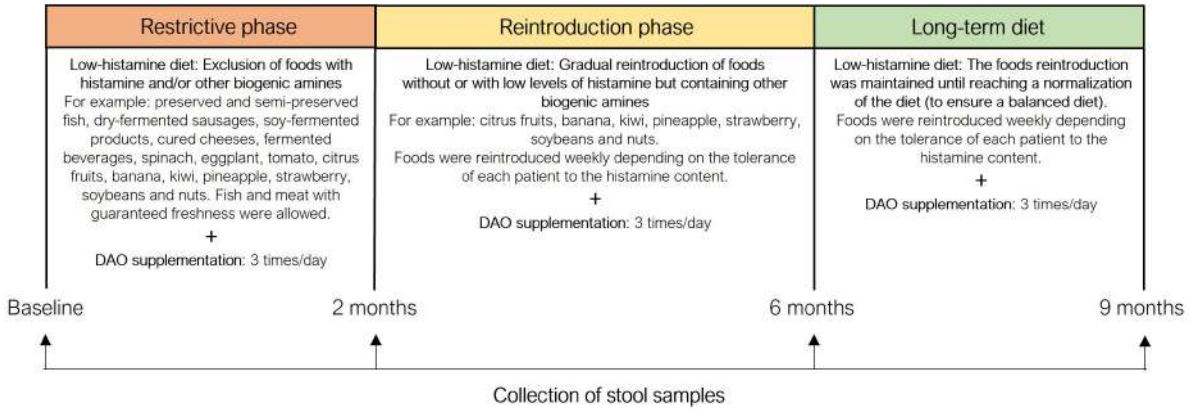

FIGURE 1
The different phases of the dietary treatment and stool sample collection during the 9-month study.

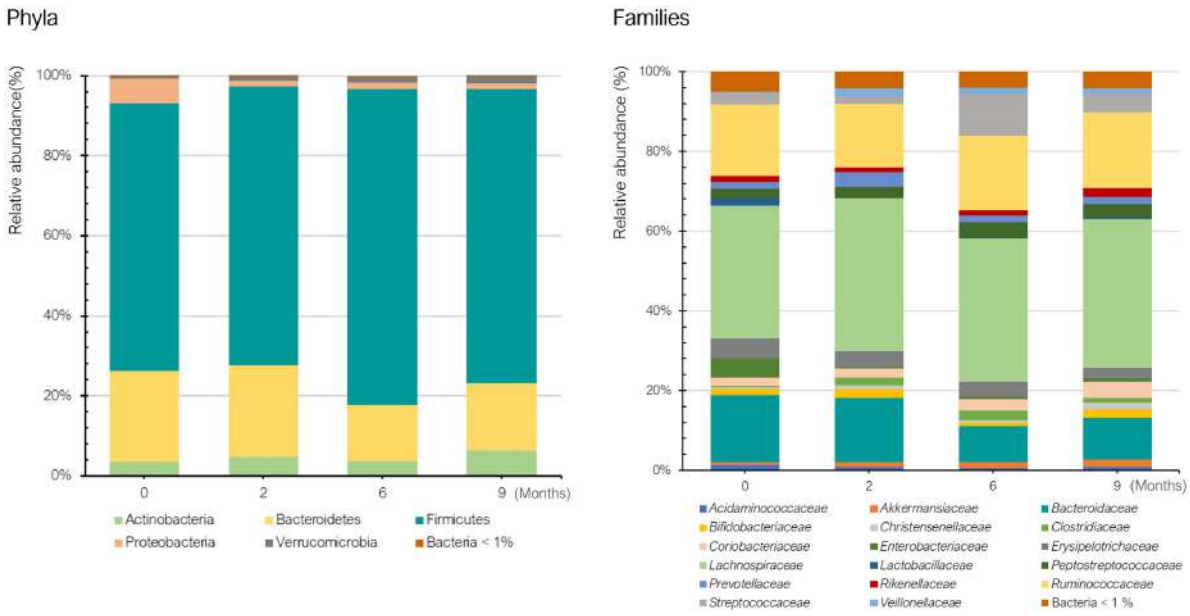

FIGURE 2
Relative abundance (%) of bacteria at phylum and family levels in histamine intolerant patients during the 9-month dietary management.

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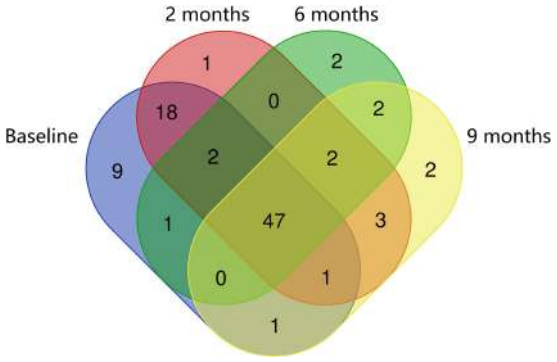

FIGURE 3
Venn diagrams of specific and shared Operational Taxonomic Units (OTUs) detected at the family level at baseline, 2, 6, and 9 months of the study.

healthy individuals used as a control group in the previous study performed by Sánchez-Pérez et al. (25). The observed statistically differences in the relative abundance of bacterial families, genera, and species are shown in **Supplementary Table 1**. In comparison with the control group, histamine intolerant patients showed a significantly higher relative abundance of some bacteria related with histamine-forming capacity (*Morganellaceae*, *Pseudomonadaceae*, *Staphylococcus*, *Proteus*, *Proteus mirabillis,* and *Clostridium perfringens*), as well as a reduction in the relative abundance of the genus *Faecalibacterium*, associated to a healthy gut (**Supplementary Table 1**).

Figure 2 shows the mean relative abundance of bacteria at phylum and family levels in the histamine intolerant patients at baseline and during the dietary treatment (low-histamine diet and DAO supplementation). A similar phylum pattern was observed for all patients at all sampling points, Firmicutes and Bacteroides having the highest relative abundance (**Figure 2**). Overall, no statistically significant differences were found in phyla during the dietary management ($p > 0.05$). The high relative abundance of the phylum Proteobacteria observed at baseline is accounted for by the abnormal abundance of these bacteria in one specific patient (24%) in comparison with the other patients (1–2.5%). It is worth highlighting that this patient (patient 3) also had the highest number of symptoms (**Table 1**). An overgrowth of Proteobacteria has been observed in patients with different intestinal disorders, such as Crohn's disease, ulcerative colitis, IBS and colorectal cancer, and has been postulated as a hallmark of dysbiosis (35–39). A marked reduction (90%) in the relative abundance of Proteobacteria was observed in this patient after 2 months of dietary treatment, when the values were similar to those of the other histamine intolerant patients. Changes in the abundance of Proteobacteria have been previously related to dietary habits (40). Levine et al. also reported a reduced relative abundance of this phylum in pediatric patients with Crohn's disease after they followed a restrictive diet (i.e., excluding foods containing wheat, dairy, animal fats, and additives) and received enteral nutrition (41).

Regarding bacterial families, the distribution pattern in the histamine intolerant patients showed few variations during the dietary treatment (**Figure 2**). Nevertheless, statistically significant changes were observed in the relative abundance of 21 bacterial families, all of them with relative abundances below 1%. These bacterial families are listed in **Supplementary Table 2**. Moreover, a reduction on the total number of families was found, decreasing from a

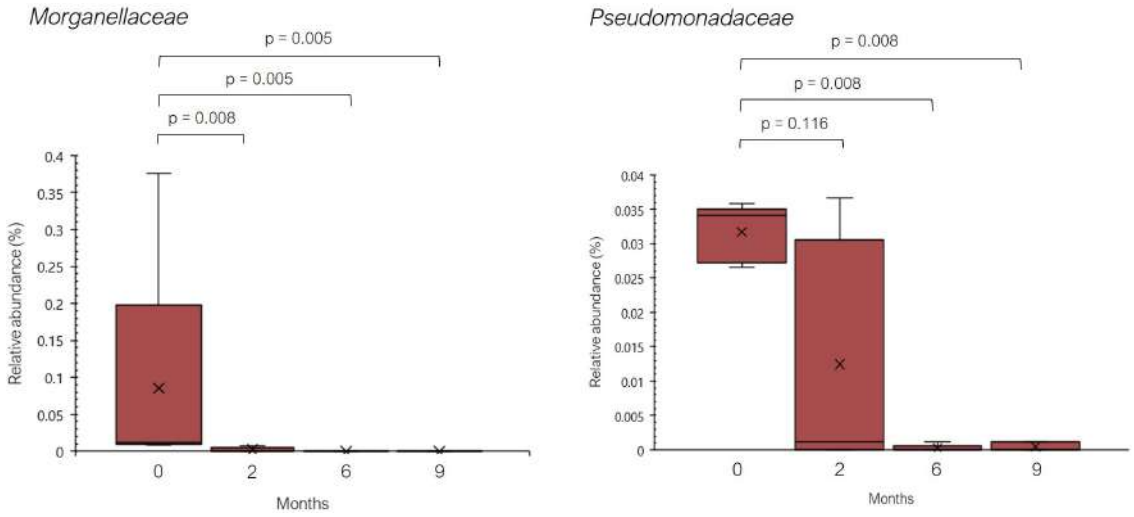

FIGURE 4
Relative abundance (%) of *Morganellaceae* and *Pseudomonadaceae* observed in histamine intolerant patients during the dietary treatment. Mean values are indicated by an ×. $p < 0.05$ indicates statistically significant differences.

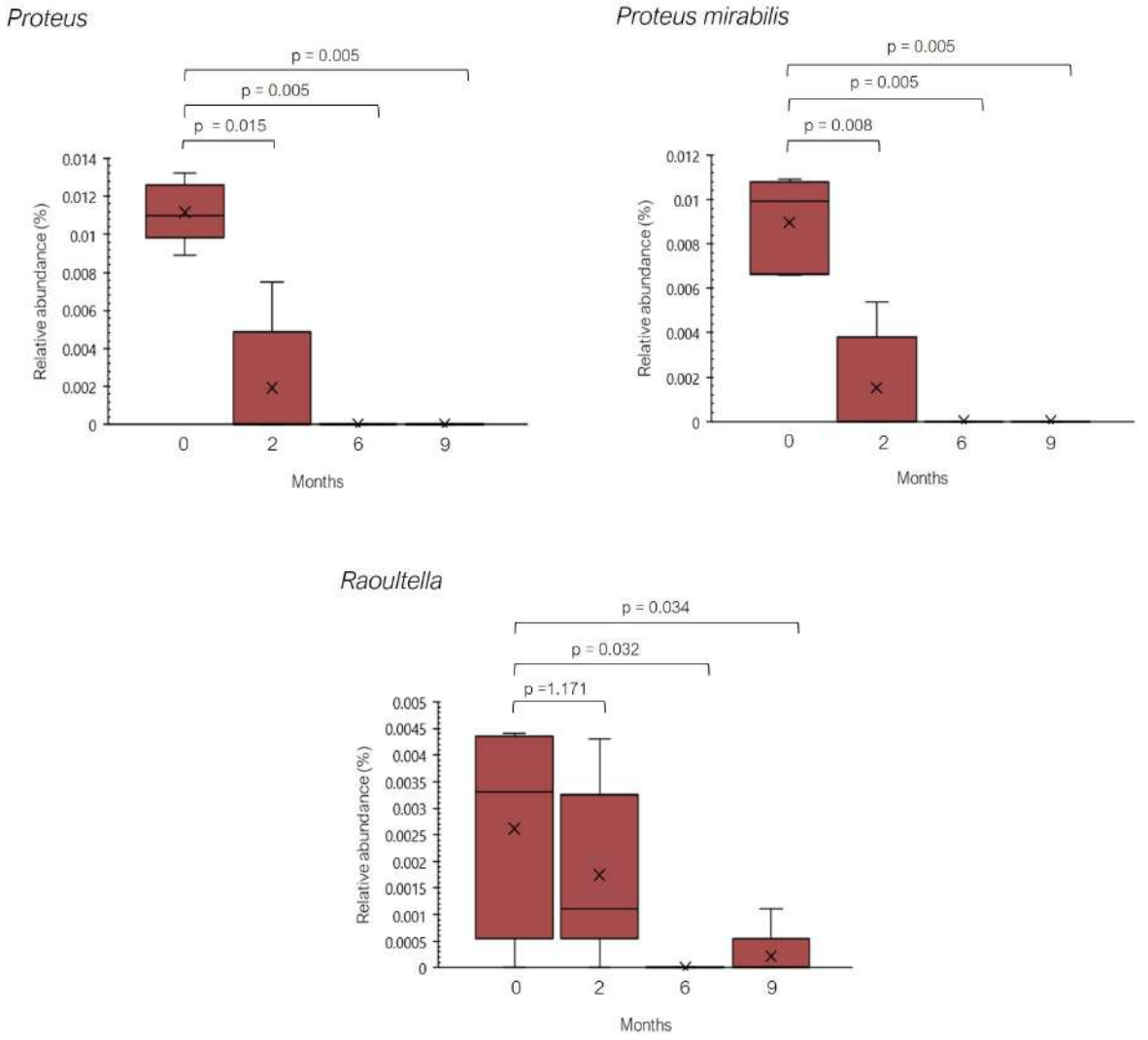

FIGURE 5
Relative abundance (%) of *Proteus*, *Proteus mirabilis,* and *Raoultella* observed in histamine intolerant patients during the dietary management. Mean values are represented with an ×. $p < 0.05$ indicates statically significant differences.

total of 79 bacterial families at baseline to 58 at 9 months, some of them related to non-desirable effects (**Figure 3** and **Supplementary Table 2**). For example, the family *Morganellaceae* was considerably reduced in all patients at the second month of the study and absent at 6 months (**Figure 4**). *Morganellaceae* includes bacterial species with a high histamine-formation capacity, such as *Morganella morganii* (29, 42–44). *Pseudomonadaceae* also showed a marked reduction in the relative abundance, especially after 6 months of dietary management (**Figure 4**). High levels of pseudomonas bacteria have been associated with cases of inflammatory bowel disease, also being a bacterial family with several strains related with histamine production (45–50).

In the previous study performed by Sánchez-Pérez et al. a greater relative abundance of several histaminogenic bacteria, mainly belonging to the family *Enterobacteriaceae* and to the genera *Staphylococcus* and *Proteus*, was reported in histamine intolerant individuals in contrast with the healthy control group (25). Thus, an excessive accumulation of bacterial-derived histamine at the intestinal level could account for the onset of symptoms associated with histamine intolerance. In the present study, statistically significant changes were observed in the relative abundance of 44 genera and 64 species during the 9-month dietary treatment (**Supplementary Tables 3**, **4**). Among them, it is worth highlighting the significant reduction observed in the relative abundance of bacteria with recognized histamine-secreting ability (29, 42–44, 51, 52). For example, the relative abundance of the genus *Proteus* and the species *Proteus mirabilis* was dramatically reduced at the second month of the study, being absent in all patients after the sixth month (**Figure 5**). For

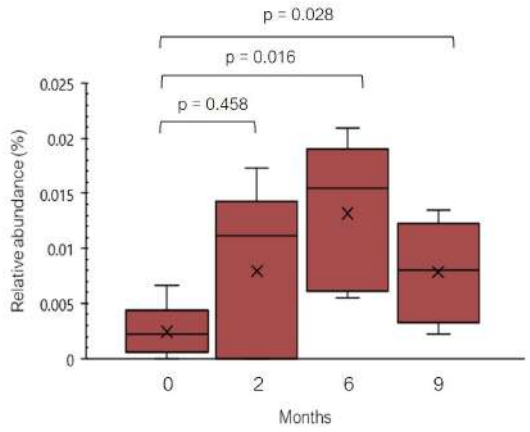

FIGURE 6
Relative abundance (%) of *Roseburia* spp. found in histamine intolerant patients during the dietary treatment. Mean values are represented with an ×. $p < 0.05$ indicates statically significant differences.

the genus *Raoultella*, this reduction was statistically significant at 6 and 9 months. In fact, Mou et al. identified some species of the genus *Raoultella* with a putative histamine-secreting capacity within the human gut microbiome (29).

On the other hand, a significant increase in the relative abundance of *Roseburia* spp. was observed throughout the dietary treatment (**Figure 6**). In addition to the reduction of histamine-secreting bacteria, it can be speculated that the decline in number of symptoms reported by the five histamine intolerant women could be partially explained by the increase in *Roseburia* spp. Beneficial properties have been attributed to *Roseburia* species due to its capacity to produce SCFA, which are involved in the maintenance of intestinal homeostasis and the inhibition of a proinflammatory status (53–55). A high abundance of SCFA-producers, among them *Roseburia*, has been found in individuals on diets in which animal-derived products are replaced with plant-based foods (56–58). Low-histamine diets tend to be richer in plant-derived products, as they aim to reduce the consumption of meat and fish derivatives and cured and raw milk cheeses.

Finally, regarding bacterial diversity, no changes were observed for any patient during the dietary treatment in terms of alpha diversity (evaluated by the Shannon and Simpson indices) ($p > 0.05$). In the case of beta diversity (assessed by Bray-Curtis dissimilarity), no significant changes were observed in interindividual differences in the distribution patterns of genera and species along the dietary treatment. In the previous work performed by Sánchez-Pérez et al. statistical differences were observed in the beta diversity between intolerant individuals and the healthy group, this latter showing more homogenous microbial patterns among them (25). In the current study, after 9 months on a low-histamine diet associated with DAO supplementation, 3 out of 5 histamine intolerant patients showed a pattern of microbial species that clustered closely with that previously reported for healthy individuals (25).

These preliminary results prompt further studies to assess the positive effect of the dietary treatment on the gut microbiota composition in histamine intolerant patients, with a larger sample set and involving healthy individuals undergoing the same dietary treatment. The lack of male representation, of data on daily food consumption, as well as regarding the changes in symptom intensity, should also be addressed in future works.

Despite the limitations of the study, preliminary results suggest that the usual dietary treatment of histamine intolerance may influence the composition of intestinal microbiota in histamine intolerant women. According to our results, a reduction in the relative abundance of histamine-secreting bacteria such as *Pseudomonadaceae*, *Proteus*, *Proteus mirabilis,* and *Raoultella* was observed during the dietary treatment. These results would support the hypothesis that a reduction in histamine-secreting bacteria could diminish the accumulation and absorption of histamine at intestinal level and, subsequently, avoid the onset of symptoms. Likewise, it was observed an increase in *Roseburia* spp., bacteria related to an improvement of the inflammation of intestinal mucosa. According to our knowledge, this is the first time that a follow-up study has been performed to assess the influence of the dietary treatment of histamine intolerance on intestinal microbiota composition. The majority of the available studies that evaluate the efficacy of a low-histamine diet on the symptoms of this intolerance considered intervention periods of up to 3 months while in this study, despite the low number of patients, 9-months of treatment were considered. Further studies with a higher number of patients are needed to better understand the relationship between the dietary treatment of histamine intolerance and changes in intestinal microbiota composition. In addition, considering the existence of certain technological bacterial strains with DAO activity it would be interesting to explore the use of DAO-positive probiotic bacteria for the preventive treatment of histamine intolerance (59).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, Bioproject PRJNA842201.

Ethics statement

The studies involving human participants were reviewed and approved by the Bioethics Committee of the University of

Barcelona (IRB00003099). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MV-N, ML-M, and MV-C: conceptualization. SS-P, OC-B, and AD: investigation. SS-P, OC-B, MB, and ML-M: data curation and writing—original draft preparation. SS-P, OC-B, AD, MV-N, MB, ML-M, and MV-C: writing—review and editing. ML-M and MV-C: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1018463/full#supplementary-material

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Smolinska, Sylwia & Winiarska, Ewa & Głobińska, Anna & Jutel, Marek. 2022. Metabolites**.**

metabolites

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Review

Histamine: A Mediator of Intestinal Disorders—A Review

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Abstract: Within the gastrointestinal tract, histamine is present at relatively high concentrations, especially during inflammatory processes. Histamine is a biogenic amine with numerous effects on many cell types, mediated by the activation of its four different histamine receptors (H1–H4Rs). It is produced and released by immune cells as mast cells and basophils. Some cells such as dendritic cells or T cells can express histidine decarboxylase, an enzyme for histamine synthesis after stimulation. The same can be done by the human gut microbiota. The production of histamine by bacteria in the human gut influence the immune response, although the major source of histamine is food. The large spectrum of histamine effects on a number of cellular processes results in various gastrointestinal disorders including food allergy, histamine intolerance, irritable bowel syndrome, and inflammatory bowel disease, among others. In this review, the protective or pathogenic effects of histamine on various gut disorders are discussed.

Keywords: histamine; histamine receptors; histamine intolerance; food allergy; inflammatory bowel disease; irritable bowel syndrome; scombroid poisoning; colorectal cancer

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1. Histamine

Histamine [2-(4-imidazolyl)-ethylamine] is a biogenic amine that was first synthesized in the early 1900s. Since then, its functions have started to be discovered and more well-described [1,2]. Histamine, which is found in many cell types, seems to be the most pleiotropic molecule in the human body [3]. The best known action of histamine is to induce contraction of smooth muscle cells (including bronchi and intestines) as well as dilate blood vessels and increase their permeability. Histamine causes heart rhythm disturbances and influences blood pressure, increases mucous secretion, gastric acid secretion, and irritation of nociceptive nerve fibers [4,5]. Histamine may also play a role in neurotransmission, immunomodulation, hemopoiesis, wound healing, intestinal ischemia, day–night rhythm regulation, and angiogenesis in tumor models [6].

1.1. Synthesis and Degradation of Histamine

Histamine is formed by oxidative decarboxylation from the amino acid L-histidine with the enzyme histidine decarboxylase (HDC). Histamine is degraded as a result of the cyclopentyl action of histamine N-methyltransferase (HNMT) and by oxidative deamination of diaminoxidase (DAO). HNMT is mainly responsible for the degradation of intracellular histamine. The highest expression of HNMT occurs in the kidneys and liver as well as in the spleen, colon, prostate, ovary, cells in the spinal cord, bronchi, and trachea [7]. A small part of histamine is converted into N-methylhistamine by the action of HNMT. In its original form, approximately 2–3% of histamine is excreted [8,9]. DAO, which is a secreted protein, is responsible for the degradation of extracellular histamine. The greatest activity of DAO is recorded in the small intestine, colon, placenta, and kidneys. The vast majority of histamine is converted into imidazole acetic acid by DAO [9].

1.2. Sources of Histamine in the Body

The main cellular source of histamine are mast cells and basophils [10]. In the Golgi apparatus of the cell, the amino acid L-histidine is decarboxylated with the enzyme L-histidine decarboxylase, whose co-factor is pyridoxal phosphate (vitamin B6). The result of this reaction is the formation of histamine, which is later stored in the cytoplasmic granules along with other amines (e.g., serotonin), proteases, proteoglycans, cytokines/chemokines, and angiogenic factors and released after sensitization and degranulation of the cell [11,12]. The degranulation of mast cells and the release of histamine occur mainly as the result of binding a specific antigen to the FcRI receptor as well as in response to non-immune stimuli (e.g., neuropeptides, parts of the complement system, cytokines, platelet activation factor). IgE antibodies are mediators of mast cell degranulation during allergic diseases. The binding of IgE to its high-affinity IgE receptor on mast cell surfaces is called "sensitization" and precedes the development of clinical allergy. Histamine released from mast cells and basophils exerts its biological activities by activating four G protein-coupled receptors, namely H1R, H2R, H3R (expressed mainly in the brain), and H4R. While H1R and H2R activation mainly accounts for some mast cell and basophil-mediated allergic disorders, the selective expression of H4R on immune cells is uncovering new roles for histamine (possibly derived from mast cells and basophils) in allergic, inflammatory, and autoimmune disorders [12]. Histamine release also results from the action of a variety of chemical and physical factors such as extreme temperatures, trauma, vibrations, or alcohol [6]. Histamine can also be synthesized and released by other cell types (e.g., gastric enterochromaffin-like cells, histaminergic neurons, dendritic cells (DCs), T lymphocytes, platelets, etc. [10]).

It is estimated that about 5% of total histamine enters the body with food or is produced by intestinal microorganisms [13]. The most popular histamine-rich foods are fish and seafood, matured or fermented foods (e.g., cheese, alcohol, pickles, etc.), and some vegetables (e.g., spinach, eggplant, tomato, etc.). According to the law in the European Union, the permissible content of histamine in food is a maximum of 200 mg/kg in fresh fish and 400 mg/kg in seafood [14]. Histidine is produced mainly in autolytic or bacterial processes, therefore high concentrations of histamine are mainly found in microbial fermentation products [15]. The conditions for the formation of biogenic amines in food are the availability of free amino acids, the presence of decarboxylase-positive microorganisms as well as the conditions enabling the growth of bacteria and the activity of decarboxylase.

Microbiota are also an important source of histamine [16–18]. The production of histamine by bacteria in the human gut has been shown to influence the immune response. Therefore, elucidating the role of histamine as a metabolite of gut bacteria is an interesting area of research. Genes encoding HDC and synthesizing histamine have been demonstrated in many Gram-positive and Gram-negative bacteria. It was shown that in bacteria belonging to the genera Lactobacillus, Pediococcus, and Oenococcus, the presence of histidine is a factor inducing the expression of genes encoding HDC, while the presence of histamine caused the opposite effect [18]. Two HDC superfamilies have been described: Gram-negative bacteria have HDCs that require the presence of a coenzyme, which is pyridoxal phosphorus. In turn, for Gram-positive bacteria, covalently bonded pyruvate is used for catalysis [19]. The secretion of decarboxylase by bacteria is regulated by many factors (e.g., the presence of fermenting carbohydrates, oxygen, or chloride concentration). In an acidic environment, the expression of the activity of amino acid decarboxylases increases. This causes a local increase in pH around the bacteria and has a protective function [20]. The species of bacteria with the highest histidine decarboxylase activity are Morganella morganii, Eschericha coli, Hafnia alvei, Proteus vulgaris, Proteus milabilis, Enterobacter aerogenes, Raoultella planticola, Raoultella ornithinolytica, Citrobacter freundii, Pseudomonas fluorescens, and Photobacterium damselae [21]. Some bacteria have the ability to metabolize histamine. The aerobic growth of Pseudomonas putida U was demonstrated on a minimal medium, the only carbon source of which was histamine. In

the six-stage catabolic process, histamine is converted into aspartic acid, which is then converted into fumaric acid. It has been shown that 11 proteins (HinABCDFLHGIJK) are necessary for the metabolism of histamine in P. Putida U. Genome studies indicate that Hin genes are present in strains of the genus Pseudomonas, but have not been shown to be present in previously sequenced Gram-positive bacteria [22]. Depending on the type of activated histamine receptor, it exerts either a pro-inflammatory or anti-inflammatory effect. Histamine derived from Lactobacillus reuteri via histamine receptor 2 inhibited the production of tumor necrosis factor-α (TNF-α) (induced by toll-like receptor) by human monocytoid cells [23]. In an experimental mice model, the immunomodulatory effect of histamine secreted by Lactobacillus rhamnosus was demonstrated. In mice without the deficiency of H2R, administration of this bacterial strain induced an anti-inflammatory effect (decreased secretion of interleukins, TNF-α) [24]. Some reports indicate that the amount of histamine secreted may determine its pathophysiological effects. These assumptions were confirmed by the study with Lactobacillus saerimneri, synthesizing almost 100 times more histamine compared to L. rhamnosus. Consequently, apart from various immunological effects, a decrease in the body weight of the animals and deterioration of the general condition were also observed [25]. The effect of bacterial secretion of histamine on intestinal diseases and digestive disorders has been reported. Therefore, it is important to deepen the knowledge of the factors influencing the synthesis, release, and metabolism of histamine by bacterial strains that make up the intestinal microbiome.

External factors that reduce the microbial diversity may cause differences in the composition of the intestinal microbiota, which may result in a state of dysbiosis. The exact mechanisms leading to dysbiosis remain unclear. The combination of physiological changes and the action of stress factors should be taken into account. Research indicates a relevant relationship between intestinal dysbiosis and the occurrence of intestinal diseases (e.g., inflammatory bowel diseases, histamine intolerance, irritable bowel syndrome) [26].

2. Histamine Scheme of Action through Histamine Receptors

The effects of histamine are due to the activation of four histamine receptor (HR) subtypes—H1R, H2R, H3R, and H4R [20,27]. Histamine receptors belong to the rhodopsin-like family of G-protein coupled receptors and are differentially expressed in numerous cell types. The tissue preference of histamine receptors are shown in Figure 1. They differ in their signaling mechanisms [28], but simultaneous activation of more than one receptor on a specific cell can lead to altered effects [29].

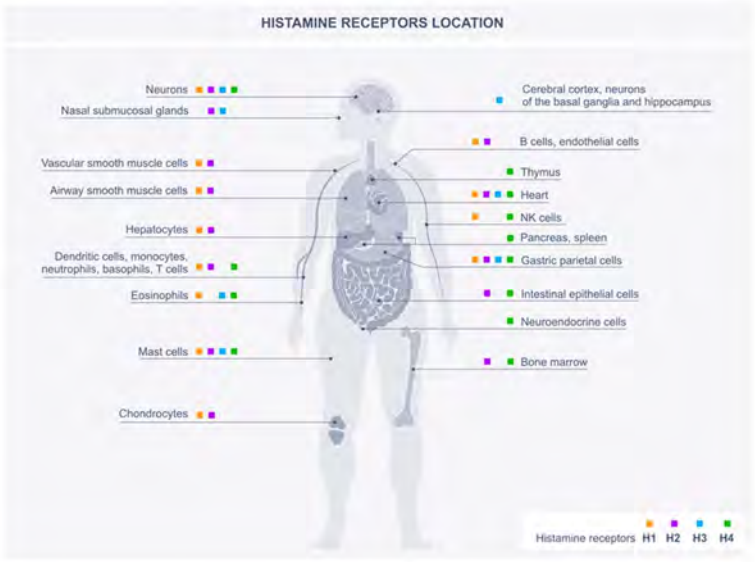

Figure 1. The location of histamine receptors in the human body.

2.1. H1R

H1 receptor activation occurs through the Gαq/11 protein, which causes the activation of phospholipase C and an increase in Ca^{2+} levels [30]. The effect of H1R activation is the contraction of airway smooth muscles, an increase in vascular permeability as well as the induction of the production of prostacyclin and platelet activating factor [31]. H1R is present in many types of cells (e.g., neurons, airway smooth muscle cells, chondrocytes, hepatocytes, endothelial cells, dendritic cells, monocytes, neutrophils, T cells, and B cells [32]). The H1 receptor is the main receptor involved in the development of allergic reactions. Allergic symptoms such as redness, itching, and swelling are related to IgE-mediated activation of mucosal mast cells. Activation of these cells results in the release of histamine and other mediators from their granularity [11]. Activation of H1R in murine models induces an increase in IFN (interferon) production, which is associated with the proliferation of type 1 T helper cells and induces a proinflammatory effect [33]. It has been shown that the expression of pruritic factors (e.g., nerve growth factor, semaphorin 3A) is regulated by histamine H1R. In murine models and patients with atopic dermatitis, the use of an H1R antagonist resulted in a reduction in IL-31 (interleukin-31) levels, which is associated with the onset of pruritus [34].

2.2. H2R

H2R expression has been observed in the digestive system (e.g., gastric parietal cells, enterocytes), smooth muscle cells, cardiomyocytes, dendritic cells (DC), and also in T and B cells. H2R receptors are postsynaptic, transmitting signals mainly via cyclic adenosine monophosphorane (cAMP) and coupling with Gαs [35]. H2R stimulation causes the external secretion of hydrochloric acid, relaxation of smooth muscle cells, and tachycardia. H2R stimulation also causes anti-inflammatory effects by inhibiting the production of IL-12, IFN-γ, TNF-α cytokines by monocytes or macrophages and mast cells, reducing the proliferation of T-helper 1 and the production of antibodies [36]. During the binding of histamine to the H2R, there is an increase in IL-10 secretion and a decrease in IL-12 levels. Consequently, DC with histamine maturation polarized naive CD4+ T cells toward the Th2 phenotype. This study suggests that Th2 cells stimulate IgE production, which may induce increased secretion of histamine by mast cells. This effect may constitute a positive feedback loop and contribute to the aggravation of atopic diseases [37]. Histamine (endogenous and exogenous) significantly changes the innate immune response to microorganisms through H2R [24]. Knockdown of H2R−/− mice caused disorders of the immune system as well as gastric defects (decreased gastric acid secretion). Cognitive decline and abnormal nociception have also been observed [38–40].

2.3. H3R

The expression of H3 receptors is observed in cells of the nervous system, especially in the cerebral cortex, neurons of the basal ganglia, and the hippocampus. H3Rs are located in the presynaptic region of histamine-containing neurons. Their function is to regulate the synthesis and release of histamine as well as other neurotransmitters (e.g., dopamine, norepinephrine, gamma-aminobutyric acid, acetylcholine, and serotonin) [41]. Changes in the expression and activation of H3 receptors play an important role in sleep–wake cycle disorders, attention deficit hyperactivity disorder, epilepsy, and cognitive disorders as well as in the development of inflammation [42]. In studies in mouse models, the induction of acetylcholine release by H3R antagonists has also been shown to increase insulin secretion as well as significantly reduce the total body weight and triglyceride levels in obese mice. This effect may be related to inducing a feeling of fullness in the hypothalamus. The hypoglycemic effect of the H3R antagonist is comparable to that of metformin [43]. H3R stimulation increases pro-inflammatory activity as well as the ability of immune cells to present the antigen. Potentially, the use of histamine H3R antagonists

could be used in preventing or inhibiting the development of inflammatory diseases (e.g., in the respiratory system) [44].

2.4. H4R

H4 receptors were discovered the most recently and their role is not yet fully understood. H4Rs are present mainly in immune cells (eosinophils, basophils, mast cells, natural killer (NK) cells, DC cells, monocytes, and T cells) and also in the spleen, thymus, bone marrow, intestinal epithelium, and neuroendocrine cells. They are also found in the bile and pancreatic ducts [45]. In contrast to other types of histamine receptors, the H4 receptor is not particularly expressed in the central and peripheral nervous system [46]. H4 receptors are coupled to Gi proteins and their downstream pathways are believed to be similar to those described for H3Rs. H4R activation is an important factor modeling chemotaxis as well as other cell functions. As a result of H4R-mediated activation of mast cells, pro-inflammatory cytokines and chemokines IL-6, TNF-α, TGF-β1 (tumor growth factor-β1), RANTES, IL-8, MIP-1α, and MCP-1 are expressed [47]. The available studies suggest that H4Rs, through interactions with Gα/io proteins, contribute to the development of inflammatory reactions and hypersensitivity [12]. Activation of H4Rs has been shown to lead to pruritus. The use of H4 antagonists had an antipruritic effect, and the simultaneous blockade of H1 and H4Rs enhanced this effect. Research suggests that H4R, by activating Th2 cells and producing IL-31, may trigger the development of allergic dermatitis [48]. Activation of H4R and H3R increases the effect of acetylcholine on the peristaltic movement of the intestines [49]. H4 receptors are also involved in peptic ulcer formation and carcinogenesis [50].

3. Histamine in the Intestines

Histamine is well-recognized for its effects in the immediate type hypersensitivity response (type I of hypersensitivity reactions by Gel–Coombs classification). The pathological effect of increased levels of histamine in the gut is less understood. However, as described further in this review article, the increased levels of histamine alter the host immune interactions with microbiota and lead to a breakdown in homeostasis, causing the development of many gut diseases that are difficult to cope with. Histamine levels in the gut can be influenced by host allergic and inflammatory responses, somehow altering the activity of enzymes that degrade or synthesize histamine as well as its dietary intake in addition to the host microbiota production. Furthermore, the amount of endogenous levels of histamine can be enhanced upon the stimulation of histamine-producing immune cells. All of this can influence gut homeostasis, lead to histamine accumulation, and breakdown to a specific disorder. The changed interactions with histamine receptors caused by their different synthesis can lead to further implications. Agonists or antagonists of histamine receptors or both added simultaneously will further modify this already complicated scenario. On top of this are environmental factors and genetic predisposition. This makes it a very complicated problem to deal with from the therapeutic point of view. Histamine may also negatively or positively influence the parasitic and bacterial infections [51,52]. All histamine receptors except H3R are also expressed throughout the intestinal tract in humans [53]. From a quantitative point of view, H4R expression is significantly less abundant in comparison to H1R and H2R, at least on the mRNA level [45,54,55].

4. Role of Histamine in Intestine Disorders

The role of histamine in intestinal disorders is schematically shown in Figure 2.

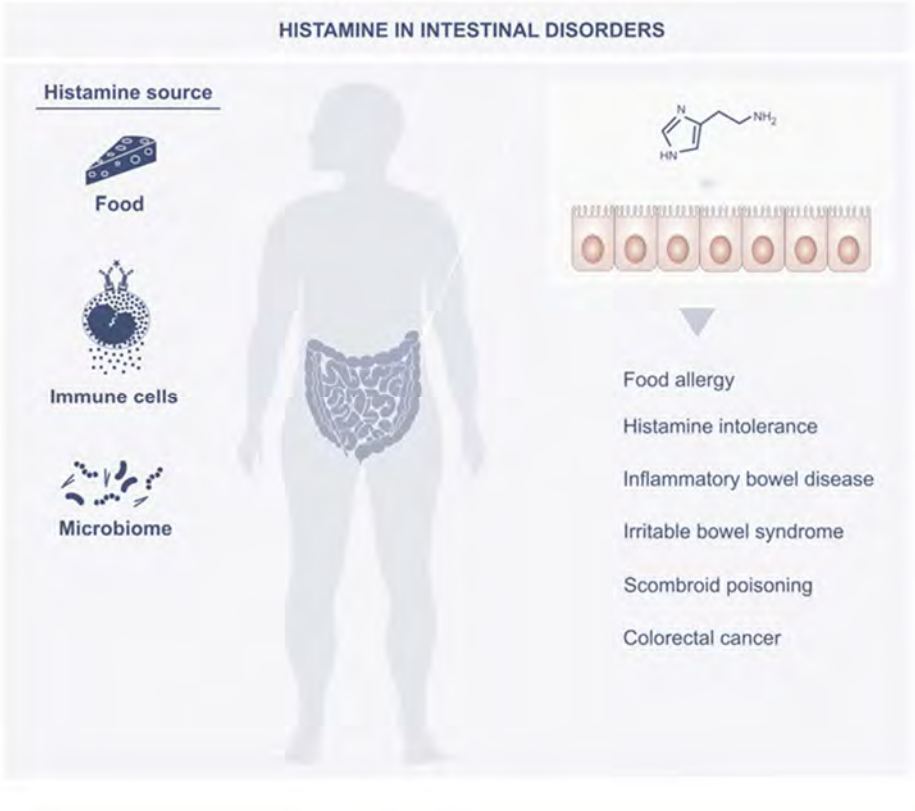

Figure 2. Histamine in intestine disorders.

4.1. Food Allergy

The digestive tract is a place that comes into contact with a large number of different molecules that are potential allergens. The characteristic symptoms of food allergies are manifested in the respiratory, digestive, cardiovascular, and skin systems. IgE-dependent food allergies develop as a result of disorders of the immune system, leading to a loss of tolerance. This leads to the recognition of mild food antigens as pathogens. Taking into account the described immunoregulatory functions of histamine, it is presumed that it may alter the immune response of the intestines to food antigens. Research results indicate the participation of histamine receptors in the development of food allergies [20]. It has been shown that the use of H2R antagonists in humans increased the production of IgE against food antigens [56]. The pathophysiology of IgE-dependent food allergy is related to the activation of the immune system. In response to a stimulus from Th2 cells, IgE binds to Fcε receptors on effector cells (mast cells and basophils). As a result of the activation of effector cells, histamine is released as well as other mediators. As a result of the IgE-dependent reaction, clinical symptoms are rapidly manifested [57]. In a food-allergic subject, enhanced secretion of histamine and increased numbers of mast cell were well-demonstrated [58–60]. For example, histamine release from basophils was positively correlated with the skin prick test, and food challenge. The anti-IgE-mediated mast cell histamine release in food-allergic patients was increased compared to the non-allergic [61]. Additionally, the incubation of biopsies from food-allergic patients with anti-IgE (human) antibodies or allergens induced a ninefold increase in histamine release. Further stimulation of biopsis ex vivo with histamine induced a concentration-dependent NO response only in the food allergic patients [62]. Food allergy can manifest as mild and severe symptoms. The most severe, potentially life-threatening manifestation is anaphylaxis. Strict avoidance of food allergens is a long-term strategy for managing IgE-mediated food allergies. Food allergy is diagnosed on the basis of clinical symptoms, skin prick tests, and the presence of specific IgE in the serum. However, the gold standard in diagnosing food allergy

is to complete the double-blind placebo controlled food challenge (DBPCFC) [63]. In this method, neither the patient nor the doctor knows what food allergen is administered. The patient is exposed to gradually increasing doses of the suspected food, hidden in a matrix. The DBPCFC can be performed in a 1- or a 2-day approach. During a 1-day approach, placebo doses containing an identical matrix are randomly interspersed. During a 2-day approach, one day consists of verum doses, and one day of the placebo. The order of the verum and placebo days are random. The DBPCFC is performed in a hospital setting, with a trained nurse and full emergency medication readily available. The challenge is discontinued when objective symptoms occur, or when consistent subjective symptoms occur on at least three subsequent doses. Due to the increasing incidence of food allergies, numerous studies have been conducted to develop new therapeutic and preventive strategies. There are also many studies on various stages of the pathogenesis of food allergies such as the influence on the Th2 pathways, blocking IgE, suppression of effector cells, and microbial therapeutics [64]. Long-term immune tolerance should be the most desirable effect in the treatment of food allergy [65]. Oral immunotherapy (OIT) is one of the developing treatments of food allergies. It consists in administering allergens to patients in doses increased every 2–4 weeks, until the maximum maintenance dose is reached. The result of this procedure is the development of tolerance to food. This method has been used in food allergies to milk, eggs, wheat, peanuts, nuts, and shellfish [66]. The FDA has approved oral immunotherapy for peanut allergy [67]. Epidermal and sublingual immunotherapy are currently under investigation. Clinical trials have also been conducted on epidermal immunotherapy in the case of allergies to milk and eggs.

4.2. Histamine Intolerance

Histamine intolerance (HIT) is a condition in which, due to the reduced ability to break down histamine, it accumulates [68]. In other words, there is no balance between accumulated histamine and the capacity for its degradation. In healthy patients, the intestinal epithelial cells have an enzyme barrier created by DAO and HMNT. This barrier prevents excessive resorption of exogenous histamine in the bloodstream. If these enzymes are inhibited or reduced, symptoms of histamine intolerance may occur after consuming even a small amount of histamine [69]. An underlying cause of histamine intolerance is diamine oxidase (DAO) deficiency, which leads to defective homeostasis and a higher systemic absorption of histamine. Impaired DAO activity may have a genetic, pharmacological, or pathological origin. The decrease in DAO activity may be caused by damage to enterocytes in the course of gastrointestinal diseases (e.g., inflammatory bowel diseases, infections). Other biogenic amines, drugs, and alcohol may inhibit the action of DAO. The microbiome also influences the development of histamine intolerance. A recent proposal also suggests that HIT can arise from an alteration in the gut microbiota [70]. A greater abundance of histamine-secreting bacteria in the gut could lead to the development of histamine intolerance. A greater number of the Bifidobacteriaceu family in healthy people has been shown. Higher numbers of the genus Proteobacteria were observed in people with decreased serum DAO activity [71].

Dysbiosis of the gut microbiota was observed in the histamine intolerance group who, in comparison with the healthy individuals, had a significantly lower proportion of *Prevotellaceae*, *Ruminococcus*, *Faecalibacterium*, and *Faecablibacterium prausnitzii*, which are bacteria related to gut health. They also had a significantly higher abundance of histamine-secreting bacteria including the genera *Staphylococcus* and *Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae*, and the species *Clostridium perfringens* and *Enterococcus faecalis*. A greater abundance of histaminogenic bacteria would favor the accumulation of high levels of histamine in the gut, its subsequent absorption in plasma, and the appearance of adverse effects, even in individuals without DAO deficiency, as the study by Sánchez-Pérez and co-authors showed [72]. Both genetic and environmental factors contribute to the development of HIT. Single nucleotide poly-

morphism of the single nucleotide polymorphism in the DAO gene results in altered production of a protein with lower enzymatic activity [8]. Increasing the amount of histamine metabolites leads to the inhibition of the second histamine metabolizing enzyme—HNMT [7]. There have also been speculation that histamine intolerance may be a metabolic disease [73]. Aside from problems with histamine digestion, a possible cause of HIT is endogenous histamine overproduction or increased exogenous ingestion of histidine or histamine from food. A plasma histamine concentration of 0.3 to 1.0 ng/mL is considered normal [74]. Common symptoms of HIT appear as a result of an increase in the levels of histamine in the body. When exposed to large amounts of histamine, even in healthy people, symptoms such as severe headache and hot flushes may occur. This effect is known as scromboid poisoning. Secondary HIT symptoms are related to the synthesis and release of catecholamines, which is caused by the increased concentration of histamine. This can cause a paradoxical increase in blood pressure, tachycardia, arrhythmias, nervousness, and sleep disturbance [6]. Symptoms of histamine intolerance are presented in Table 1.

Table 1. Symptoms of histamine intolerance from a specific organ.

	Symptoms of Histamine Intolerance
Respiratory system	rhinorrhea, rhinitis, nasal congestion, dyspnea, sneezing
Cardiovascular system	tachycardia, hypotonia, collapse
Gastrointestinal system	bloating, flatulence, postprandial fullness, diarrhea, abdominal pain, constipation, nausea, vomiting
Reproductive system	menstrual cramps, dysmenorrhea
Skin	pruritus, flushing, urticarial, dermatitis, swelling
Nervous system	headache/migraine, dizziness, chronic inappropriate fatigue, nervousness, sleep disturbances (insomnia), anxiety, panic disorder, depression

Clinical diagnosis of HIT remains a challenge, as standardized diagnostic tests are lacking. The diagnosis of histamine intolerance can be made only after excluding other causes that may produce similar symptoms. IgE-mediated food allergy, mastocytosis, and the action of drugs that may interfere with the metabolism and distribution of histamine should be ruled out. Diagnosis usually requires the presence of at least two clinical symptoms in less than four hours after food intake and their improvement or remission after a low-histamine diet. Complementary tests such as the determination of DAO activity in blood samples or intestinal biopsy and the identification of genetic and metabolic markers are also available [8,75]. The gold standard of treatment is a low-histamine diet. A good response to 4–8 weeks of such a diet is considered to confirm the diagnosis of histamine intolerance. DAO supplementation is also recommended as a complementary treatment in people with intestinal DAO deficiency [75,76]. In severe conditions where a low-histamine diet is insufficient, H1R antihistamines can be used for a short time. Some studies have shown that supplementation with DAO enzyme cofactors such as vitamin C, copper, and vitamin B6 may be an adjunctive therapy [77]. An interesting field of research seems to be supplementation with probiotic microorganisms. Such an approach could lead to a reduction in the production of the bacterial L-histidine decarboxylase enzyme as well as to the simultaneous degradation of histamine (or other biogenic amines). There are no studies assessing the effectiveness of supplementation with probiotic microorganisms. However, based on the available information, it can be assumed that members of the genus Bifidobacterium may be considered as candidates for adequate supplementation [71,78].

4.3. Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD) are idiopathic, chronic-recurring diseases of the gut. Their two main manifestations, ulcerative colitis (UC) and Lesniowski–Crohn's dis-

ease (CD), differ in their clinical, endoscopic, and histologic appearance. In CD, the inflammation appears in diffuse lesions that can be found all over the digestive tract and deeply penetrates the intestinal wall, possibly affecting all layers. In contrast, inflammatory lesions in UC start in the rectum, proceed upward but do not exceed the colon, and remain superficial at the mucosa. Lesniowski–Crohn disease leads to transmural inflammation throughout the whole gastrointestinal tract but is characterized by a discontinuous pattern. In contrast to ulcerative colitis where inflammation is superficial, ulcerations are limited mainly to the colon mucosa. IBD decreases the quality of the patients' lives and not treated, can be life threatening. Both manifestations present similar symptoms (e.g., mucosal lesions, ulcers, edema, diarrhea, bloody stool, abdominal pain). Studying the available knowledge about the mechanisms of these diseases, it is easy to conclude that both disease development is a result of complex interactions between the host immune system, enteric microbiota, and environmental factors in genetically susceptible patients. Mucosal histamine levels (not plasma levels) are increased in patients with IBD. Increased levels of N-methylhistamine that correlated with disease activity were found in the patients' urine [59,79,80]. Mast cells originating from the resected colon of active Lesniowski–Crohn's disease or ulcerative colitis were able to release more histamine than those from the normal colon when being stimulated with an antigen, colon-derived murine epithelial cell-associated compounds [14]. Similarly, cultured colorectal endoscopic samples from patients with IBD secreted more histamine toward substance P alone or substance P with anti-IgE than the samples from normal control subjects under the same stimulation [15]. The histamine signaling pathway is disrupted in both patients with Lesniowski–Crohn disease and ulcerative colitis, as shown in the detailed analysis conducted by Smolinska and co-authors [81]. Histamine receptor expression and functional activity is altered in IBD patients. In addition blockage of H2R resulted in more severe inflammatory disease in the murine T-cell transfer colitis model. Histamine mostly suppresses IFN-γ and TNF-α secretion, and the gene expression of these cytokines correlates positively with H4R and H2R expression accordingly in patients with ulcerative colitis. HNMT gene expression is reduced in inflamed mucosa, and DAO polymorphisms have been associated with an increased risk of IBD [81–83]. The use of the H2R antagonist (but not proton pump inhibitors) increases the risk of hospitalization or surgery in Lesniowski–Crohn disease patients [42]. Transfer of T cells, which lack H2R or inhibit H2R using femotidine (H2R antagonist), accelerates weight loss and increases the disease severity in a mouse colitis model. Patients with IBD are treated with anti-inflammatory drugs, steroids, antibiotics, aminosalicylates, or welcome biological therapy with the use of infliximab (anti-TNF-α). In many cases, the only option to obtain a state of remission is radical surgery, where the inflamed areas are cut out. Potentially, the use of an antagonist for H1R and H4R with the simultaneous use of the H2R agonist can be beneficial for patients with IBD [81].

4.4. Irritable Bowel Syndrome

Functional dyspepsia (FD), irritable bowel syndrome (IBS), and small intestinal bacterial overgrowth (SIBO) are commonly reported, but solely as symptom-oriented conditions. These clinical syndromes continue to be imprecise and were therefore re-named to "IBS-like" disorders [84]. There is a lack of specificity of symptoms. Abdominal pain, diarrhea, nausea, vomiting, etc. are general symptoms linked with gastrointestinal disorders. IBS is a chronic condition linked to abdominal discomfort or pain where the food eaten is a trigger of more severe symptoms. Some evidence has shown that the gastrointestinal microbiota is altered and perhaps this disrupted mucosal immune response plays a significant role [85,86]. In one study, more than half of the patients experienced gastrointestinal symptoms from histamine-releasing food items and foods rich in biogenic amine [87]. The level of endogenous histamine definitely correlates with the severity of symptoms in IBS patients. Activated mast cells produced higher amounts of histamine, which correlated with abdominal pain in IBS patients [88]. Mucosal biopsy supernatants

from IBS patients contained higher levels of histamine compared to supernatants delivered the same way from healthy subjects [89,90]. Histamine levels and the abundance of HDC genes were determined in both healthy and IBS patients using metabolomics and metagenomics data from the integrative Human Microbiome Project. These analyses revealed that IBS patients presented higher levels of histamine and bacterial HDC genes [91]. Subsequent studies have also shown that supernatants from colonic samples of IBS patients contained increased histamine levels, and the expression levels of histamine receptors H1R and H2R were upregulated in IBS patients [92]. The authors thus hypothesized that a dysbiosis with increased histamine-secreting or HDC-containing bacteria was potentially associated with the development and aggravation of IBS [93]. Administration of specific microbes has therapeutic effects, which can also be an argument for microbiota changes as a cause of disease [94–96]. There is no specific treatment for IBS. Drugs that decrease inflammation are in use.

4.5. Scombroid Poisoning

Scombroid poisoning or histamine fish poisoning results from mishandled fish [97]. The symptoms are variable and can include oral numbness, headache, dizziness, palpitations, low blood pressure, difficulties in swallowing, week or rapid pulse, hives, rush, flushing, swelling of face, vomiting, nausea, and diarrhea. Histamine is generated in fish tissue by bacterial conversion of free histidine by a wide range of bacterial species: Morganella morganii, Enterobacter aerogenes, Raoultella planticola, Raoultella ornithinolytica, and Photobacterium damselae. Aside from high levels of histamine, its toxicity can be enhanced by inhibitors of enzymes that degrade histamine. DAO and HNMT inhibitors were also present in the ingested fish [98]. In addition, toxins that induce mast cell degranulation are found in spoiled fish, leading to further increases of histamine in the host body [99]. Furthermore, some substances that have the potential to be histamine receptor blockers were also found in the fish. The symptoms of scombroid disease are usually rapid and do not last longer than 24 h. Treatment includes the administration of antihistamines.

4.6. Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related mortality [100]. Patients with inflammatory bowel disease have an increased lifetime risk of CRC compared with the general population [101,102]. This risk can be reduced by the treatment of colitis with the suppression of intestinal inflammation [103]. The role of the intestinal microbiome in colon cancer development has been investigated [104–107]. Specific gut microbes and their metabolites may contribute to the cause of CRC [108–110]. Histidine decarboxylase deficiency has been shown to promote inflammation-associated colorectal cancer by the accumulation of $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cells, indicating a potential antitumorigenic effect of histamine. Several probiotic strains including *Bifidobacterium longum* [111], *Lactobacillus acidophilus* NCFM [112], and *Lactobacillus rhamnosus* GG [113] have shown beneficial effects in different murine models of colon cancer. This histamine-producing probiotic decreased the number and size of colon tumors and the colonic uptake of [^{18}F]-fluorodeoxyglucose by positron emission tomography in *HDC*$^{-/-}$ mice. Administration of *L. reuteri* suppressed keratinocyte chemoattractant (*KC*), *Il22*, *Il6*, *Tnf*, and *IL1α* gene expression in the colonic mucosa and reduced the amounts of proinflammatory, cancer-associated cytokines, keratinocyte chemoattractant, IL-22, and IL-6, in plasma. Histamine-generating *L. reuteri* also decreased the relative numbers of splenic $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cells. Furthermore, an isogenic HDC-deficient *L. reuteri* mutant that was unable to generate histamine did not suppress carcinogenesis, indicating a significant role of the cometabolite, histamine, in the suppression of chronic intestinal inflammation and colorectal tumorigenesis. In the colonic mucosa of CRC patients, HDC activity as well as histamine content were increased in comparison to the normal samples [114,115]. However, in mice with experimentally induced CRC, the deletion of HDC resulted in enhanced tumorigenesis compared to the wild type mice,

pointing toward an anti-carcinogenic effect of histamine, which is supported by the finding that gut microbiota-derived histamine suppresses colorectal tumorigenesis [116]. On one hand, histamine promotes the underlying inflammatory process, leading to tumor initiation. On the other hand, histamine in the tumor's tissue may affect the differentiation of immature myeloid cells toward neutrophils and myeloid-derived suppressor cells, both resulting in tumor regression [117,118]. While the effect of histamine on the differentiation toward neutrophils is a direct one, the differentiation of myeloid-derived suppressor cells is affected by IL-17, which is produced by tumor-associated mast cells upon histamine stimulation. This anti-cancer effect of histamine is supported by similar findings obtained in models of esophageal squamous carcinoma [119]. Interestingly, mast cells have been found to be abundant in colon carcinoma and to promote carcinogenesis in chemically-induced CRC in mice [120], and are associated with a poor prognosis in human CRC patients [121]. In analogy to the pro-inflammatory effect of histamine via the H4R, the absence of H4R expression also leads to a reduction in chemically-induced carcinogenesis in mice [122]. However, some indications arise from the observation that the expression of H4R decreases in gastric carcinoma during progression, accompanied by the attenuated histamine-induced suppression of proliferation [119,123].

5. Conclusions

Histamine is a mediator that is mainly recognized due to its role in inducing allergic symptoms, but it is also involved in non-allergic inflammatory reactions. The role of histamine present within the gastrointestinal mucosa is of special interest. Its potential seems to be underestimated. In some concentration ranges, histamine plays a protective role and is pivotal to maintain the healthy status. However, at higher concentrations histamine contributes to the pathophysiology of mucosal inflammatory disorders. The overlap of various mechanisms complicates the understanding of its role in disease and the possible design of diagnostics and curative modalities based on them. The application of various medications that utilize mechanisms interfering with histamine signals could be beneficial for patients. Enhancement of H2R expression and/or its intracellular signals with simultaneous decrease H1R or/and H4R activity is a plausible approach to improve mucosal immunity including a protective umbrella in both allergy and autoimmunity. The use of gut microbiota with the potential to release histamine offers a novel therapeutic perspective.

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Intestinal Dysbiosis in Patients with Histamine Intolerance

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Abstract: An underlying cause of histamine intolerance is diamine oxidase (DAO) deficiency, which leads to defective homeostasis and a higher systemic absorption of histamine. Impaired DAO activity may have a genetic, pharmacological or pathological origin. A recent proposal also suggests it can arise from an alteration in the gut microbiota, although only one study has explored this hypothesis to date. A greater abundance of histamine-secreting bacteria in the gut could lead to the development of histamine intolerance. Thus, the aim of this study was to characterize the composition of the intestinal microbiota of patients with histamine intolerance symptoms and compare it with that of healthy individuals. The study was performed by sequencing bacterial 16S rRNA genes (V3-V4 region) and analyzing the data using the EzBioCloud Database. Dysbiosis of the gut microbiota was observed in the histamine intolerance group who, in comparison with the healthy individuals, had a significantly lower proportion of *Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* and *Faecablibacterium prausnitzii*, which are bacteria related to gut health. They also had a significantly higher abundance of histamine-secreting bacteria, including the genera *Staphylococcus* and *Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae* and the species *Clostridium perfringens* and *Enterococcus faecalis*. A greater abundance of histaminogenic bacteria would favor the accumulation of high levels of histamine in the gut, its subsequent absorption in plasma and the appearance of adverse effects, even in individuals without DAO deficiency.

Keywords: histamine; histamine intolerance; gut microbiota; intestinal dysbiosis; histamine-secreting bacteria; diamine oxidase (DAO) enzyme

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1. Introduction

In the last several years, there has been growing interest in characterizing the gut microbiota, both in healthy and unhealthy individuals. It is well known that the eubiotic gut microbiota has an impact on human health and well-being [1]. Although its composition is age-related and becomes more stable in adulthood, it can be altered by a wide range of factors, such as dietary habits, lifestyle, stress, antibiotic use and diseases [1,2]. While the connection between the gut microbiota and certain noncommunicable diseases, such as obesity, diabetes, cancer, gastrointestinal and neurological disorders, is being extensively studied, its role in food intolerance, including that of histamine, is still under explored [3–9].

Histamine intolerance is an adverse reaction to dietary histamine that appears in susceptible individuals [10,11]. This disorder is mainly provoked by a deficiency in the key enzyme responsible for histamine degradation at the intestinal level, diamine oxidase

(DAO), which leads to higher absorption [12–14]. The accumulation of histamine in plasma can affect numerous organs and tissues due to the wide distribution of the four histamine receptors in the organism, resulting in a plethora of gastrointestinal and extra-intestinal symptoms (i.e., dermatological, respiratory, neurological and hemodynamic complaints). According to the retrospective study by Schnedl et al. (2019), the most common symptoms in histamine-intolerant patients are gastrointestinal in nature, above all abdominal distension, postprandial fullness, diarrhea, abdominal pain and constipation [15].

DAO deficiency may have a genetic origin and has been associated with single-nucleotide polymorphisms encoding a protein with reduced histamine degradation capacity [16,17]. On the other hand, impaired DAO activity can also be temporary and reversible, arising as a side effect of some widely used pharmacological drugs, such as clavulanic acid or acetylcysteine, or a secondary symptom of gastrointestinal disorders [18]. In fact, evidence supporting an intestinal origin of histamine intolerance is growing [3]. A group of Austrian researchers found that the mucosal damage caused by gastroenteritis, irritable bowel syndrome (IBS), short bowel syndrome or gastrointestinal surgery led to a concomitant decrease in DAO and lactase activities [19]. Moreover, recent studies have suggested that reduced DAO activity may be linked to nonceliac gluten sensitivity [20–22]. Another cause of DAO deficiency could be an alteration in the composition of the gut microbiota, although to date only one study has explored this hypothesis [23]. Schink et al. (2018) reported that the intestinal dysbiosis in patients diagnosed with histamine intolerance could contribute to mucosal inflammation, a condition that impairs DAO activity [23]. Additionally, the fact that a range of bacterial strains in the human gut are able to produce (*Enterococcus faecalis*, *Bifidobacterium pseudocatenulatum, Lactobacillus gasseri, Escherichia coli, Morganella morganii* and *Proteus mirabillis*) and degrade (*Escherichia coli* and *Klebsiella pneumoniae*) histamine suggests that dysbiosis could influence histamine levels in the intestine [24]. In this context, the aim of this work was to characterize the composition of the gut microbiota of patients with symptoms of histamine intolerance and compare it with that of healthy individuals.

2. Materials and Methods

2.1. Study Design

The study was carried out with 26 volunteers, including 12 patients diagnosed with histamine intolerance (HIT group), who were recruited from a nutrition and dietetic centre specialized in the dietary management of DAO deficiency (DAO Deficiency Clinical Institute, Barcelona, Spain). The inclusion criteria for the histamine-intolerant patients were as follows: age between 18 and 65 years; diagnosis of histamine intolerance based on two or more symptoms described by Maintz and Novak (2007) [25]; and negative results for food allergen-specific IgE. The exclusion criteria were pregnancy, lactation, having started a low-histamine diet and having taken antibiotics and/or probiotics the month before the study. The 14 healthy individuals in the control group, who were free of histamine intolerance symptoms, were recruited from the Food and Nutrition Campus of the University of Barcelona.

Demographic characteristics and clinical symptoms of all study participants were recorded. For the intestinal microbiota and histamine concentration analysis, walnut-sized stool samples were self-collected in sterile vials and stored at −80 °C until their analyses. For the HIT group, plasma DAO activity was also analyzed using a Radio Extraction Assay (REA) according to the manufacturer's instructions (Sciotec Diagnostic Technologies, Tulln, Austria).

All participants were informed in detail about the aim and procedure of the study and gave their written informed consent prior to study inclusion. The study was approved by the Bioethics Committee of the University of Barcelona (IRB00003099).

2.2. Intestinal Microbiota and Histamine Concentration Analysis in Stool Samples

Bacterial DNA was isolated from stool samples using a QIAamp Power Fecal Pro DNA kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. DNA concentration was measured by BioDrop μLite (Biotech, Madrid, Spain). To analyze the composition of the gut microbiota, sequencing of the V3-V4 region of bacterial 16S rRNA was performed on the Illumina MiSeq platform by the Genomic and Bioinformatic Service of the Autonomous University of Barcelona. Then, bioinformatics analysis of the microbiota composition was performed with EzBiocloud Database (ChunLab, Inc., Seoul, Korea). For the 16S rRNA amplicons, sequence data were deposited on the NCBI database by the Bioproject PRJNA811749.

Stool histamine was determined by a competitive enzyme linked immunoassay using the Histamine ELISA kit from Immunodiagnostik AG (Bensheim, Germany) according to the instructions provided by the manufacturer.

2.3. Statistical Analysis

Statistical analyses of participant characteristics and the histamine concentrations in stool samples were performed using the IBM SPSS Statistics 25.0 statistical software package (IBM Corporation, Armonk, NY, USA), applying Student's t or Mann–Whitney tests after the Kolmogorov–Smirnov test for normal distribution. Differences in the microbiota composition between groups were analyzed by the Kruskal–Wallis test for non-parametric data. Alpha diversity was measured using the Shannon index and Simpson's index, and, for beta diversity, Bray–Curtis dissimilarity analysis was performed and visualized using principal coordinates analysis (PCoA). p-values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Participant Characteristics

All participants in the HIT group were female and aged between 21 and 65 years (mean 40.4 ± 12.4). In the control group, the volunteers were 71.4% female and 24–55 years old (40.4 ± 12.4), and no significant differences were observed between the sexes in any of the study parameters (Table 1).

Table 1. Characteristics of the participants from the control and HIT groups.

Participants' Characteristics	Groups	
	Control	HIT
n (%)	14 (53.9%)	12 (46.2%)
Age (average years ± SD)	40.4 ± 12.5	40.4 ± 12.4
Male [*n* (%)]	4 (28.6%)	0 (0%)
Female [*n* (%)]	10 (71.4%)	12 (100%)
Body Mass Index (BMI) [average ± SD]	23.7 ± 3.2	22.2 ± 6.0

The symptoms described by histamine-intolerant patients are summarized in Table 2. Gastrointestinal and neurological disorders were reported by 83% of patients with histamine intolerance, followed by dermatological (50%) and respiratory complaints (33%). The mean number of symptoms per patient was 4.3, although it was striking that two patients reported 7 and 8 symptoms, respectively. Overall, the most frequently reported symptoms were bloating and headache, followed by flatulence; diarrhoea; heart burn; and abdominal, muscular and articular pain. These were also the most common symptoms in histamine-intolerant patients identified by Schendl et al. (2019) in a cohort of 133 individuals [15]. It is worth mentioning that approximately half of the patients in the present study were underweight, with body mass index (BMI) values below 18.5.

Table 2. Clinical manifestations reported by the HIT group (*n* = 12).

Symptoms	Frequency (%) *
Gastrointestinal tract	
Bloating	75
Flatulencies	33
Abdominal pain	25
Diarrhoea	25
Heartburn	25
Constipation	17
Nausea	17
Skin	
Urticaria	17
Atopic skin	17
Pruritus	8
Eczema	8
Neurologic system	
Headache	75
Dizziness	8
Respiratory apparatus	
Asthma	17
Rhinitis	8
Shortness of breath	8
Other symptoms	
Muscular/articular pain	25
Fatigue	17
Insomnia	8

* The frequency (%) refers to the number of patients suffering these symptoms within the HIT group.

DAO plasmatic activity was deficient in 10 out of the 12 patients with symptoms of histamine intolerance (<10 U/mL). Although DAO plasmatic activity has been proposed as a potential marker of histamine intolerance, its reported prevalence varies greatly (values ranging from 8% to 88%), depending on the study and the symptoms [26]. Discrepancies in the data could be explained by the variable etiology of DAO deficiency, which may be genetic in origin, a secondary symptom of gastrointestinal pathologies, or arise from the consumption of DAO-inhibitor drugs.

3.2. Intestinal Microbiota Composition and Stool Histamine Concentration

The intestinal microbiota of HIT and control groups was analyzed and compared in terms of phylum, family, genus and species. The two study groups shared a similar profile of phyla (Figure 1a), with Firmicutes and Bacteroidetes being the most dominant (approximately 90% of the total gut microbiota). Although without statistical significance, the HIT group showed a slightly higher relative abundance of the phylum Proteobacteria (3.52%) in comparison with the control group (1.88%). Similarly, Schink et al. (2018) reported higher levels of Proteobacteria in patients with histamine intolerance symptoms. According to this work, the intestinal overgrowth with Proteobacteria could result in a low-grade intestinal inflammation that could lead to epithelial dysfunction. High proportions of this phylum have also been found in patients with different intestinal disorders, such as Crohn's disease, ulcerative colitis, colorectal cancer and IBS [27–30]. Intestinal inflammation may increase the amount of oxygen available in the intestinal lumen, resulting in a shift from obligate anaerobic bacteria towards facultative anaerobic bacteria, such as Proteobacteria. Consequently, an increase in its abundance has been postulated as a hallmark of dysbiosis [31,32].

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Figure 1. Relative abundance (%) of bacteria at the level of (**a**) phylum, (**b**) family, (**c**) genus and (**d**) species in control and histamine intolerance (HIT) groups. The genera and species are only represented if differences between the study groups were significant.

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Regarding bacterial families, *Lachnospiraceae* (Firmicutes), *Ruminococcaceae* (Firmicutes) and *Bacteroidaceae* (Bacteroidetes) represented more than 50% in both the control and HIT groups (Figure 1b). Statistically significant differences between groups were observed in four bacterial families ($p < 0.05$) (Table 3). For example, a lower abundance of *Prevotellaceae* (Bacteroidetes) was found in the HIT group. An under-representation of several members

of this family group may indicate reduced mucin synthesis, which has been associated with increased gut permeability [33]. Schink et al. (2018) reported higher mean values of a marker of intestinal permeability (zonulin) in histamine intolerance patients in comparison with those recommended for the healthy population, suggesting a mild alteration of gut permeability in these patients [23]. According to these authors, an increased gut permeability facilitates the penetration of microbial metabolites, such as histamine, and, in turn, could lead to histamine-associated symptoms. Additionally, bacteria belonging to the *Prevotellaceae* family have been associated with a range of functions in the organism, such as interaction with the immune system and the synthesis of thiamine, folate and short-chain fatty acids [33].

Table 3. Differences in the relative abundance (%) of bacterial families between the control and HIT groups. Data are presented as average ± SD.

Family	Phylum	Control	HIT	*p*-Value
Acholeplasmataceae	Tenericutes	0.001 ± 0.002	0.002 ± 0.001	0.03
Actinomycetaceae	Actinobacteria	0.012 ± 0.011	0.027 ± 0.024	0.02
Prevotellaceae	Bacteroidetes	7.422 ± 7.996	1.548 ± 2.408	0.04
Staphylococcaceae	Firmicutes	0.002 ± 0.007	0.014 ± 0.029	0.03

All the genera and species identified in the microbiome of both study groups are shown in the Supplementary Material (Supplementary Tables S1 and S2). Overall, statistically significant differences were found in the relative abundance of 21 genera and 30 species between control and HIT groups ($p < 0.05$) (Figure 1c,d). In their study with histamine-intolerant patients, Schink et al. (2018) reported significant differences in five bacterial genera, including only those with an abundance greater than 0.01% [23]. Applying the same criterion, in the present study, more differences were identified at the genus level (up to nine).

Although the relative abundance of *Ruminococcus* in the control group was highly variable, it was statistically significantly lower in individuals with histamine intolerance ($p < 0.05$) (Figure 2). *Ruminococcus* is thought to play a role in maintaining a healthy human gut [34]. Members of this genus can degrade complex polysaccharides into a variety of simple sugars, making them more available for the epithelium cells of the large intestine [34,35]. The relative abundance of the genus *Faecalibacterium* (Figure 2), especially the species *Faecalibacterium prausnitzii* (Figure 3), was also significantly lower in the HIT group ($p < 0.05$). Proposed as a marker of gut health, *F. prausnitzii* is one of the most prevalent and abundant producers of butyrate in the human gut, a short-chain fatty acid that represents the main energy source for colonocytes, and it displays protective properties against colorectal cancer and inflammatory bowel diseases [36–38]. Regarding *Bifidobacterium* and *Lactobacillus* (Figure 2), two other bacterial genera frequently associated with good intestinal health, no significant differences were found between the two groups. Only two species displayed a lower mean relative abundance in the HIT group (*Bifidobacterium adolescentis*, $p = 0.034$ and *Lactobacillus rogosae*, $p = 0.017$) (Supplementary Table S2).

Conversely, the genera *Staphylococcus* and *Proteus* were significantly more abundant in the HIT group ($p < 0.05$), with mean values 7- and 1.8-fold higher than in the control group, respectively (Figure 2). Several bacteria from these genera have shown an important capacity to form histamine [39–41]. Moreover, members of the family *Enterobacteriaceae*, known to be among the most prolific histamine-producing bacteria, were also significantly more abundant in the HIT group (Figure 2) [39–41], although they could not be identified at the genus level. It should be mentioned that the ability to form histamine is reported to be strain-dependent [42].

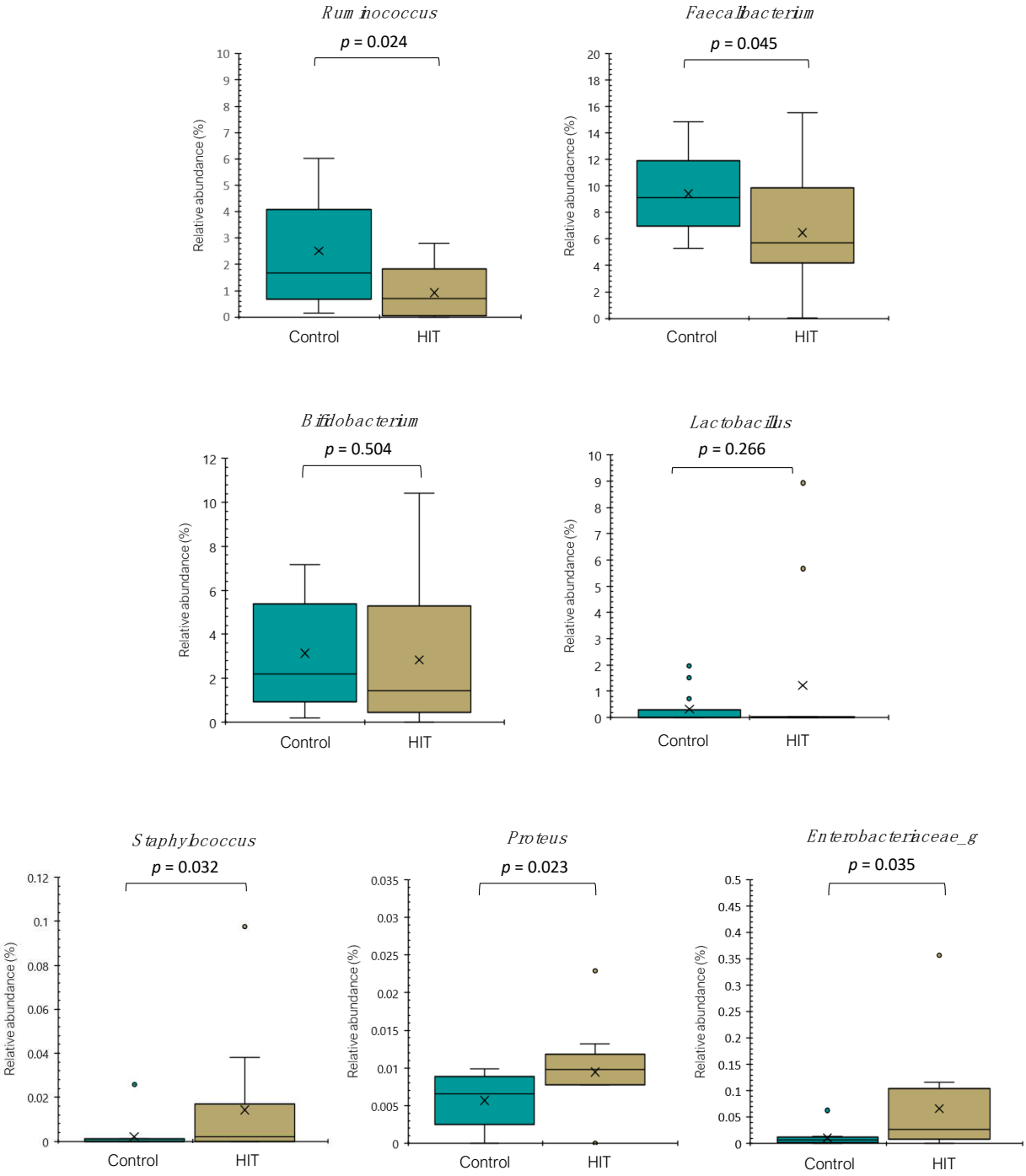

Figure 2. Relative abundance (%) of different genera in the control and HIT groups. Mean values are represented within each box and values statistically considered as outliers (atypical values) are plotted as circles.

To date, studies on histamine-producing bacteria have been mainly focused on strains isolated from food samples. However, the histaminogenic capacity of the gut microbiota has been studied only recently, and data are still limited [32,43,44]. A systematic in silico search published in 2021 identified 117 species with a putative histamine-secreting capacity within the human gut microbiome [32], many of them belonging to genera extensively reported as histaminogenic, such as *Morganella*, *Lactobacillus*, *Staphylococcus*, *Photobacterium* and *Clostridium* [24,41,42]. For example, according to Mou et al. (2021), *Clostridium perfringens* is one of the species most frequently associated with the enzyme histidine decarboxylase, regardless

of strain [32]. In the present study, the occurrence of *C. perfringens* (Figure 3), a bacterium responsible for several gastrointestinal disorders, was more frequently identified in the HIT group and only in two healthy individuals. Similarly, the abundance of *Enterococcus faecalis*, *Proteus mirabilis* and *Escherichia coli* tended to be higher in the HIT group (Figure 3). These species were isolated from the human gut by Pugin et al. (2017) and identified as producers of histamine as well as other biogenic amines, such as putrescine, cadaverine and tyramine [24].

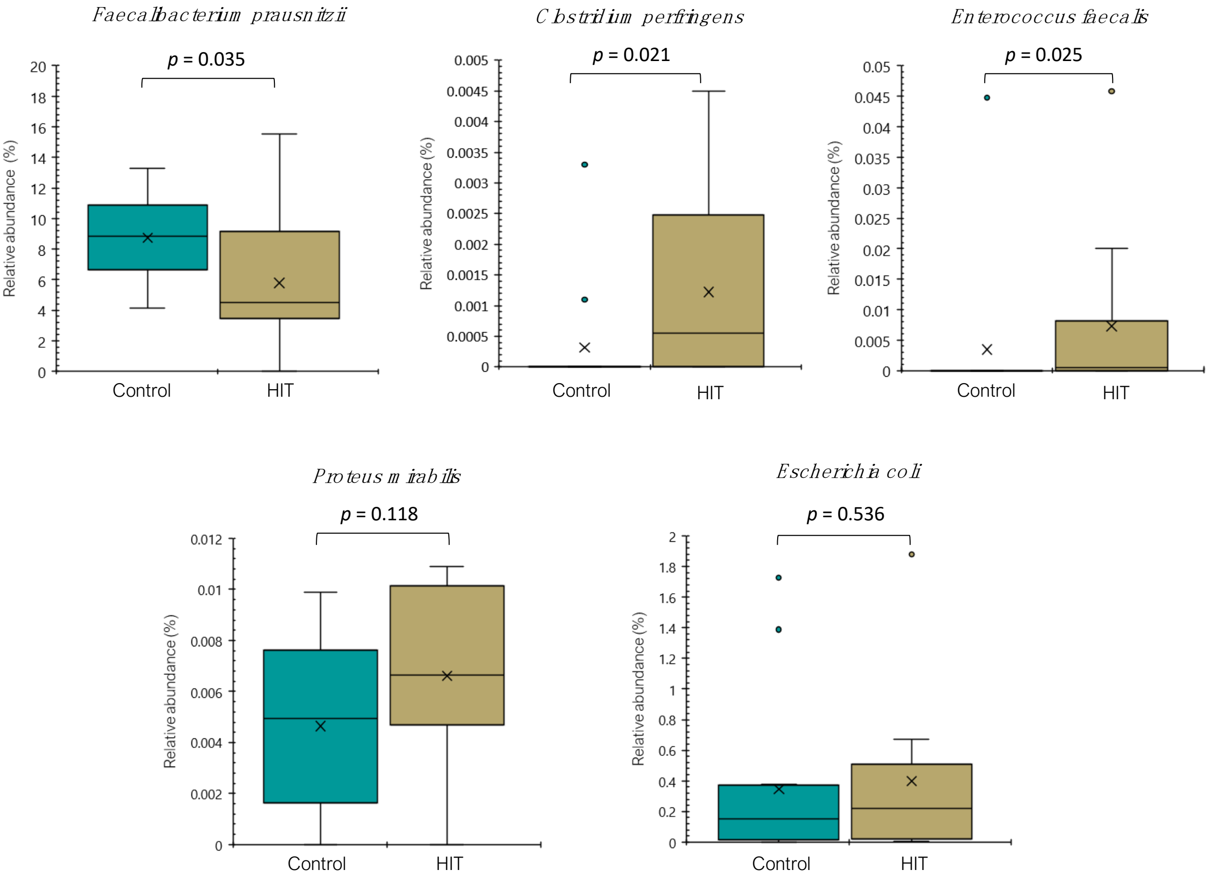

Figure 3. Relative abundance (%) of different species in the control and HIT groups. Mean values are represented with an × and values statistically considered as outliers (atypical values) are plotted as circles.

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It has been suggested that histamine secreted by the gut microbiota could have an impact on the health or disease status of the host. In the present study, the higher abundance of intestinal histaminogenic bacteria found in histamine-intolerant patients could have resulted in an excess accumulation and systemic absorption of histamine. Notably, patients with histamine intolerance frequently suffer from DAO deficiency, which could also enhance the toxicity of intestinal histamine. Additionally, an excess of histamine could negatively affect the inflammatory state of the intestinal mucosa. High amounts of histamine secreted by gut bacteria were linked to a proinflammatory response and signs of deteriorating health in murine specimens [43,45,46]. Mishima et al. (2020) suggested that intestinal dysbiosis, involving an over-representation of histamine-secreting bacteria and higher intestinal histamine levels, was potentially associated with the development and aggravation of IBS [47]. After analyzing 2451 stool metagenomes, Mou et al. (2021) also found that putative histamine-secreting bacteria were significantly enriched in patients with ulcerative colitis and Crohn's disease [32]. According to these authors, the enrichment of histamine-secreting species in IBD patients was not attributed to a single taxon but highly dependent to the cohort characteristics. The involved bacterial taxons included Actinobac-

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Concerning the histamine concentration in stools, no significant differences were found between study groups ($p = 0.681$). As shown in Figure 4, the majority of both healthy and histamine-intolerant individuals (71% and 92%, respectively) displayed fecal histamine levels within the normal range (<959 ng/g stool). The obtained results are in accordance with those of Schink et al. (2018), who also found very similar histamine levels among stool samples [21–23]. Therefore, the increased presence of histamine-secreting bacteria found in the HIT group was not associated with higher histamine excretion in feces.

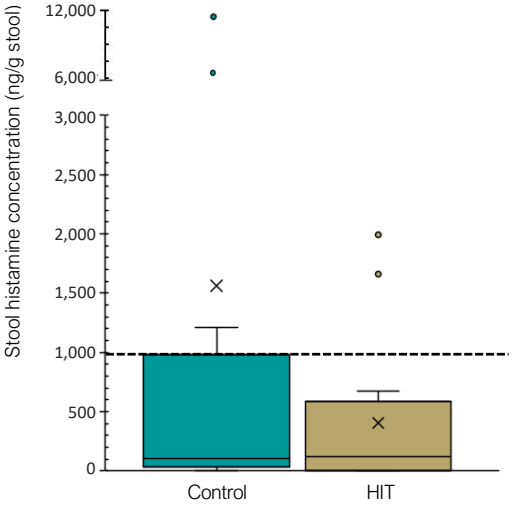

Figure 4. Occurrence of histamine (ng/g stool) in fecal samples of control and HIT groups. Samples above the dotted line are above the normal range (<959 ng/g stool). Mean values are represented with an × and values statistically considered as outliers (atypical values) are plotted as circles.

3.3. Bacterial Diversity

Bacterial species diversity was evaluated through indices of alpha diversity (Shannon and Simpson indices) and beta diversity (multidimensional scaling by PCoA and Bray–Curtis dissimilarity). Regarding alpha diversity, which is a measurement of the mean species diversity within the human gut, no significant differences were observed between the HIT and control groups for any of the evaluated indices (Shannon index, $p = 0.411$ and Simpson index, $p = 0.681$). Figure 5 shows the number of identified species belonging to the main genera that differed significantly in abundance between the two groups. Although the HIT group showed a significantly different proportion of genera with the capacity to form histamine (*Staphylococcus* and *Proteus*) and genera considered as a biomarker of a healthy gut (*Ruminococcus* and *Faecalibacterium*), these differences were not observed in terms of species number. However, a lower diversity in *Bifidobacterium* and *Lactobacillus* species was observed in the HIT group, with individuals showing only 69% and 59% of the species found in the control group, respectively (Figure 5).

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In disagreement with our results, Schink et al. (2018) found a lower alpha diversity in a group of 8 histamine-intolerant individuals in comparison with 10 healthy subjects [23]. Similar discrepancies exist in studies on other types of food intolerance or gastrointestinal disorders, some observing a reduced alpha diversity in patient groups [51–53] and others reporting no differences in this parameter [54,55].

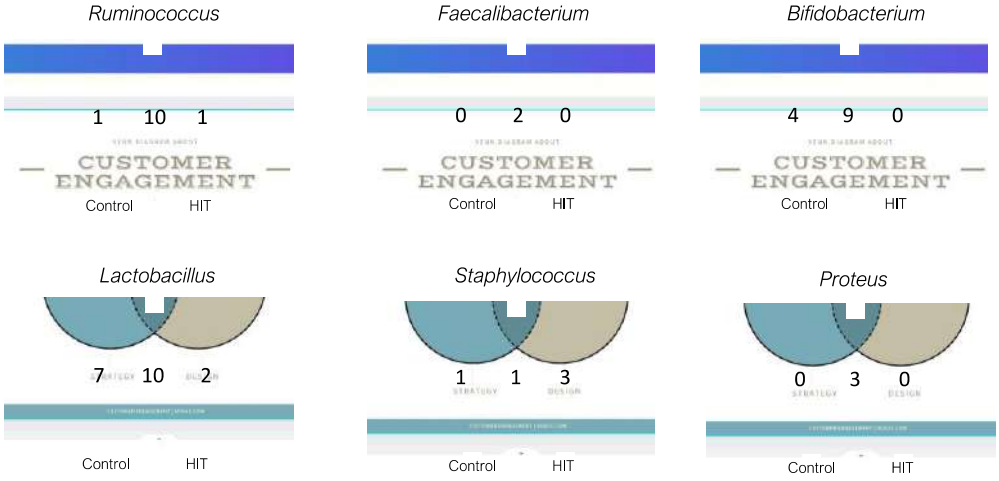

Figure 5. Venn diagrams of specific and shared Operational Taxonomic Units (OTUs) detected for *Ruminococcus*, *Faecalibacterium*, *Bifidobacterium*, *Lactobacillus*, *Staphylococcus* and *Proteus* genera in the control and HIT groups.

Beta diversity refers to the interindividual differences in the distribution pattern of genera and species. In this case, beta diversity determined by the Bray–Curtis index showed statistically significant differences between the two groups, both for genera (p = 0.024) and species (p = 0.029). As shown in Figure 6, the samples of the HIT group are more scattered compared to those of healthy individuals, which denotes a higher degree of heterogeneity in their intestinal microbiota.

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(a)

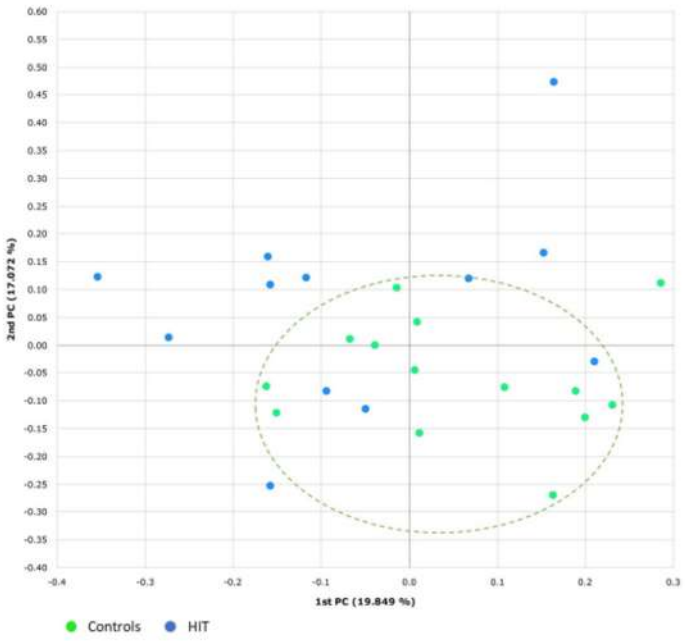

Figure 6. *Cont.*

(b)

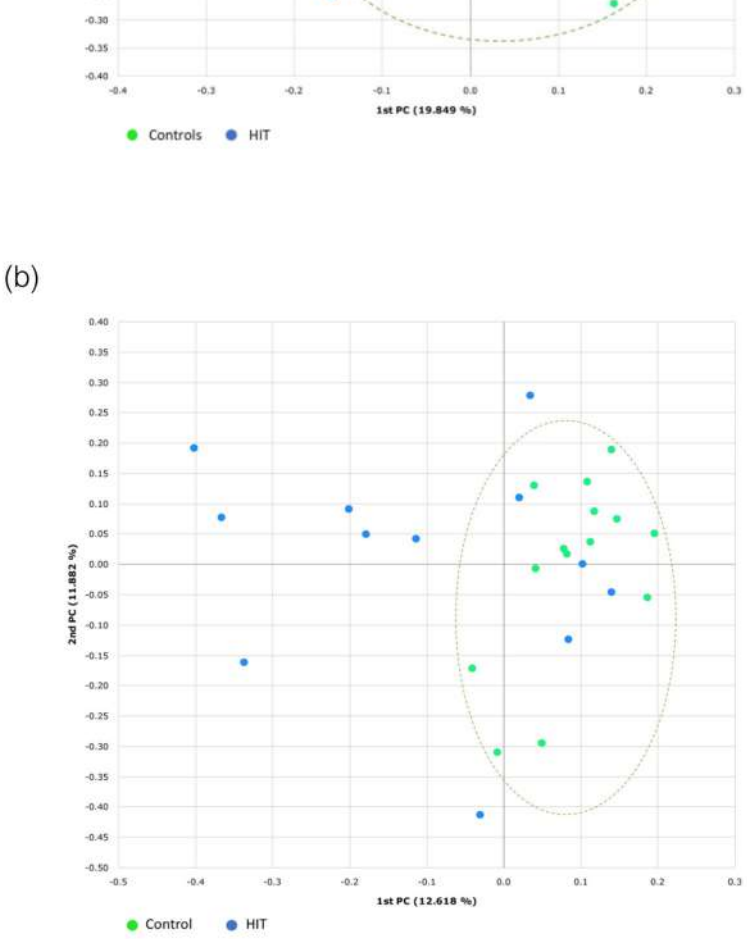

Figure 6. Beta diversity at the (**a**) genus and (**b**) species level determined by the Bray–Curtis index and principal coordinates analysis (PCoA). PC in both axes means "principal component".

4. Conclusions

An imbalance or dysbiosis of the gut microbiota was observed in patients with histamine intolerance in comparison with healthy individuals. In the HIT group, the relative abundance of bacteria associated with gut health, namely *Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* and *Faecablibacterium prausnitzii*, was significantly lower, whereas that of histamine-secreting bacteria was significantly higher, including the genera *Staphylococcus* and *Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae*, and the species *Clostridium perfringens* and *Enterococcus faecalis*. A greater abundance of histaminogenic bacteria may favor the accumulation of high levels of histamine in the gut and its subsequent absorption in plasma, which can trigger adverse health effects. The ability to degrade histamine derived from an intestinal dysbiosis would be easily overwhelmed in individuals with DAO deficiency. This dysbiosis could also provoke mucosal inflammation, a condition that impairs DAO functionality. Therefore, an over-representation of histamine-forming bacteria in the gut could be another possible origin of histamine intolerance.

The main limitations of the present study are the small size of each sample group, the lack of male representation in the HIT group, and in some cases, the impossibility of classifying bacteria beyond the family or genus level. Moreover, the fact that approximately half of the participants from the HIT group showed a reduced BMI may also be considered as a drawback of this study as it could be another factor influencing the gut microbiota composition. These limitations should be born in mind in further studies aimed at elucidating the relationship between intestinal dysbiosis and histamine intolerance. Next steps should focus on the development of studies with a more ambitious design considering a higher number of participants to better understand until what extent an imbalance in the presence of histamine-secreting bacteria or gut health-related bacteria would be etiologically linked with the symptomatology of histamine intolerance. Moreover, it would also be of interest to assess the potential influence of the follow-up of a low-histamine diet in the intestinal microbial pattern of histamine-intolerant individuals.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14091774/s1, Table S1: Bacterial genera obtained in the characterization of the intestinal microbiota in control and HIT groups; Table S2: Bacterial species obtained in the characterization of the intestinal microbiota in control and HIT groups.

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Relationship between allergic rhinitis and diamine oxidase activity: A preliminary report.

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Relationship between allergic rhinitis and diamine oxidase activity: A preliminary report

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Abstract. Aim: To analyze the diamine oxidase (DAO), the main catabolic enzyme of histamine, degradation activity and its relation with symptoms of persistent allergic rhinitis. Methods: In this descriptive and analytical observational study, we collected DAO activity levels and the nasal peak inspiratory flow. Results: Enzymatic activity deficit in 108 patients was 46.3% (95% CI, 0.44 – 0.63), 33.33% in mild and 47.92% in moderate/severe rhinitis (p = 0.376). The nasal peak inspiratory flow in patients with a deficit in DAO activity was 76.30 ± 28.40 L/min compared to 93.62 ± 37.50 L/min in patients with normal enzymatic activity (p = 0.010). Conclusions: It seems that the lower the catabolic activity of DAO, the lower the nasal peak inspiratory flow observed. Although DAO activity levels could be a severity biomarker in allergic rhinitis, a cause-effect association cannot be concluded. The enzyme could be another actor in the pathophysiology of allergic rhinitis.

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Introduction

Allergic rhinitis (AR) is an inflammation of the mucous lining clinically defined by nasal symptoms induced by an immunologically mediated reaction after the exposure of the nasal mucous membranes to an offending allergen. These symptoms include rhinorrhea, nasal obstruction, nasal itching, sneezing, and postnasal drip [1].

Histamine is the main mediator, producing nasal airway exudation, itching, and obstruction in subjects with AR through an immune reaction [2, 3, 4]. Mast cells and vascular endothelial cells synthesize and store it from the decarboxylation of the amino acid L-histidine, and its catabolism is regulated mainly by diamine oxidase (DAO). In mammals, this enzyme is expressed in specific tissues, especially the gastrointestinal tract, placenta, and kidney [2, 5, 6]. Under normal circumstances, DAO forms an enzymatic barrier in cells of the intestinal epithelium, which sufficiently protects from resorption of histamine from ingested food into the blood stream [2, 5, 6, 7].

A failure in the function of this enzyme results in an imbalance of the ingested histamine and the capacity for its degradation [2, 5, 6]. Some clinical studies have correlated DAO deficiency with specific pathologies, mainly gastrointestinal, dermatological diseases or migraine [6, 8, 9, 10, 11, 12, 13, 14, 15], depending on the expression of histamine receptors (H1 – H4) in tissues [16]. However, there is little information available about DAO function in patients diagnosed with AR and its correlation with symptoms

[17, 18, 19]. The aim of this study is to analyze the DAO degradation activity in patients with persistent AR.

Methods

Study design and data collection

This was a descriptive and analytical observational cross-sectional study in adult patients diagnosed with persistent AR, recruited from the outpatient departments of the Otorhinolaryngology, Head and Neck Surgery Department of a tertiary hospital. Selection of the volunteer participants was performed through a structured interview conducted by one Otorhinolaryngologist and one Allergologist. This research involved human participants and was approved by the Hospital's Ethics Committee (Code: 2016/106). Written informed consent was obtained from all individual participants included in the study.

Inclusion criteria were to have a confirmed diagnosis of persistent AR according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines criteria [1], to not present important nasoseptal deformities or polyps objectivated by physical examination (anterior rhinoscopy and flexible fibronasoendoscopy, and to have not been treated for the past 2 weeks with DAO enzyme or any medication that could not be suspended and causes acquired decrease (antihistamines, systemic glucocorticosteroids, aminophylline, cefuroxime, clavulanic acid, metoclopramide, verapamil, etc.) or increase (heparin) in DAO activity [2, 5, 7]. Additionally, patients with a 2-week intake history of foods containing histamine such as fermented foods, beverages, processed meat, and seafood, or patients with any other medical disorders (hepatic, gastrointestinal, or renal disease, deficiency of vitamin B6, vitamin C, copper, or zinc, etc.) or pregnant were excluded from this study [2, 5, 7].

Sociodemographic variables, DAO enzyme activity, the health-related quality of life ESPRINT-15 questionnaire [20], the degree of severity according to the ARIA guidelines (mild and moderate/severe) [1], and the nasal peak inspiratory flow (NPIF) were collected [21, 22].

ESPRINT-15 questionnaire

The ESPRINT-15 (Cuestionario ESPañol de Calidad de Vida en RINiTis) is a specific quality of life questionnaire for allergic rhinitis, validated in the Spanish population and with reference values that facilitate its proper interpretation. It has 15 items in 4 dimensions (symptoms, daily activities, sleep, and psychological well-being), with scores from 0 to 6. Lower scores indicate better quality of life [23]. Reference values in persistent AR were (men: mild = 0.5, moderate/severe = 2.6; women: mild = 0.8, moderate/severe = 2.7) [20, 22, 24].

Nasal peak inspiratory flow

NPIF is a simple and rapid technique that is carried out using a plastic tube (20 cm long, 3 – 4 cm in diameter, and calibrated at 30 – 370 L/min) to which a face mask is attached (GM Instruments Ltd., Irvine, UK). From an expiratory maneuver to residual volume, a forced inspiration is made while the lips are sealed. Three measurements that must not vary by more than 10% are taken, and the best one is chosen. Normal values for adult males (Caucasian) were 143 ± 48.6 L/min and for adult females (Caucasian) 121.9 ± 36 L/min [21, 22].

DAO activity analysis

Blood samples were collected from all subjects by venipuncture in an EDTA tube after an 8-hour fasting period. Samples were analyzed with ELISA to determine DAO enzyme activity in accordance with the manufacturer's instructions (D-HIT, Sciotec Diagnostic Technologies GmbH, Tulln an der Donau, Austria). This method was previously used for the same purpose by Mušič et al.[25] Values above 80 HDU/mL (histamine degrading Unit/mL) were considered normal, while values below 80 HDU/mL were considered DAO deficient. One HDU corresponds to the DAO activity that degrades 1 pmol/mL of histamine.

Statistical analysis

Statistical analysis was performed with the statistical package R 3.6.1 (The R Foun-

Table 1. Diamine oxidase enzyme activity values and nasal peak inspiratory flow according to groups.

		HDU/mL			L/min		
	N (%)	Mean ± SD	Median	p-value	Mean ± SD	Median	p-value
Population	108	91.20 ± 40.81	84.14	–	85.60 ± 34.55	85	
DAO activity deficit	50 (46.3)	59.77 ± 11.19	59	**< 0.000**	76.30 ± 28.40	75	**0.010**
Normal DAO activity	58 (53.7)	118.29 ± 37.49	105.27		93.62 ± 37.50	90	
Men	36 (33.33)	96.66 ± 37.88	92.09	0.101	100.83 ± 36.42	95	**0.001**
Women	72 (66.67)	88.47 ± 42.19	77.09		77.98 ± 31.13	77.5	
Severity groups							
Mild	12 (11.11)	110.22 ± 52.07	92.09	0.118	107 ± 32.85	100	**0.016**
Moderate/Severe	96 (88.89)	88.83 ± 38.87	83		82.91 ± 33.97	80	
Mild							
Normal DAO activity	8 (66.67)	133.57 ± 48.86	121.18	**0.008**	120 ± 33.17	112.5	**0.031**
DAO deficit	4 (33.33)	63.52 ± 3.28	63.52		81.25 ± 7.5	80	
Moderate/severe							
Normal DAO activity	46 (47.92)	115.85 ± 35.35	105.27	**< 0.000**	89.4 ± 36.71	85	0.057
DAO deficit	50 (52.08)	59.44 ± 11.59	57.68		75.86 ± 29.53	72.5	

DAO = diamine oxidase; HDU = histamine degrading unit; SD = standard deviation. **Bold** = statistically significant.

dation for Statistical Computing, Vienna, Austria). Statistical tests were two-tailed with a 95% confidence interval (CI). A minimum sample size of 97 randomly selected subjects was calculated to estimate, with a precision ± 8% units, a population DAO deficiency percentage considered to be ~ 20%, based on previous literature [6, 11, 17]. Normality was evaluated by the Kolmogorov-Smirnov test and variances using the Levene test. Quantitative variables were expressed as mean ± standard deviation (SD) and median. The comparison of means between groups was performed using the Student's t, Mann-Whitney, ANOVA, or Kruskal-Wallis test as appropriate. Qualitative variables were expressed as frequency and percentage. The differences between groups were evaluated by the χ^2-test, Fisher's exact test or its variants as appropriate. In cases where non-normality was significant, non-parametric methods were applied (Wilcoxon test and Kruskal-Wallis test).

Results

Descriptive analysis

A total of 108 Caucasian patients with persistent AR were recruited, 36 (33.33%) men and 72 (66.67%) women. The mean age was 32.91 ± 12.8 years, 34.15 ± 11.79 years for men and 32.28 ± 13.31 years for women (p =0.323), respectively. 64.15% of the population were non-smokers, and the remaining percentage was divided between smokers (n = 18; 16.98%) and ex-smokers (n = 20; 18.87%), with no gender differences (p = 0.070). According to the ARIA severity of the symptoms, 12 (11.11%) patients had mild rhinitis (4 men and 8 women), and 96 (88.89%) had moderate/severe rhinitis (32 men and 64 women), with no gender differences.

DAO enzyme activity

The mean blood determination of the DAO enzyme activity was 91.20 ± 40.81 HDU/mL, being 96.66 ± 37.88 HDU/mL in men and 88.47 ± 42.19 HDU/mL in women (p = 0.101). The prevalence of DAO activity deficit was 46.3% (95%CI, 0.44 – 0.63, p < 0.000). The mean activity was 118.29 ± 37.49 HDU/mL in the group with normal function compared to 59.77 ± 11.19 HDU/mL in the group with deficit (p < 0.000).

Depending on the rhinitis severity, the mild group had a prevalence of DAO deficit of 33.33% compared to the moderate/severe group, with a 47.92% (p = 0.376). The mean DAO activity in patients with mild rhinitis was 110.22 ± 52.07 HDU/mL compared to 88.83 ± 38.87 HDU/mL of patients with moderate/severe rhinitis (p = 0.119). Considering the smoking habit, no differences were

Table 2. Typical nasal symptoms and histamine intolerance-related symptoms.

	Normal activity N (%)	DAO deficit N (%)	OR	p-value
Nasal symptoms				
Anosmia	38 (65.52)	26 (56.52)	0.68	0.349
Nasal Obstruction	50 (86.21)	44 (95.65)	3.52	0.105
Sneezing	54 (93.1)	44 (95.65)	0.15	0.580
Itching	44 (75.85)	46 (100)	**0**	**< 0.000**
Posterior rhinorrhea	36 (62.07)	26 (56.52)	0.79	0.567
Anterior rhinorrhea	48 (82.76)	38 (82.61)	0.98	0.984
Hit symptoms				
Migraine > 2/month	6 (10.34)	12 (25)	**2.89**	**0.041**
Constipation/diarrhea	18 (31.03)	26 (54.17)	**2.62**	**0.016**
Gastrointestinal discomfort	22 (37.93)	30 (62.5)	**2.72**	**0.012**
Musculoskeletal pain	46 (79.31)	34 (70.83)	0.63	0.313
Skin problems	12 (20.69)	10 (20)	0.96	0.432
Generalized fatigue	30 (51.72)	20 (41.67)	0.67	0.302
Fibromyalgia	2 (3.45)	2 (4.17)	1.22	1
Asthma	22 (37.93)	20 (41.67)	1.17	0.695

OR = odds ratio; HIT = histamine intolerance. **Bold** = statistically significant.

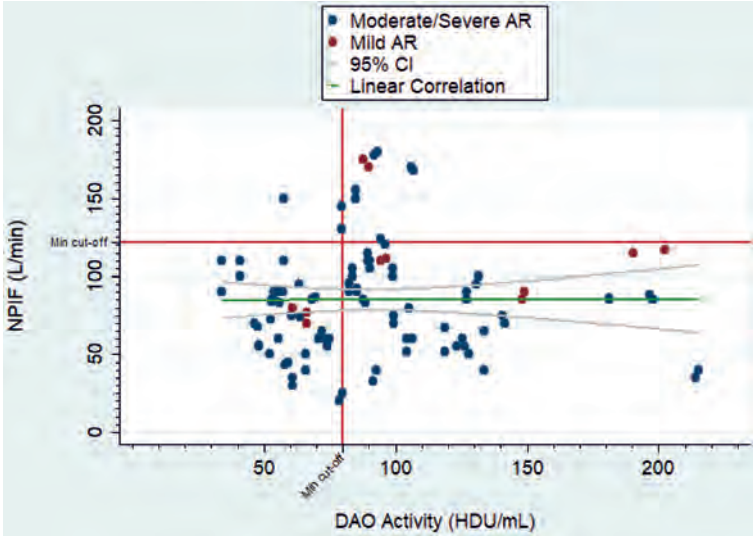

Figure 1. Correlation between diamine oxidase activity levels and nasal peak inspiratory flow according to allergic rhinitis severity group. The minimum cut-off points for both measurements are indicated.

found in DAO activity levels (p = 0.518) or the prevalence of DAO activity deficit (p = 0.089). The rest of the activity values are described in Table 1.

Nasal peak inspiratory flow

The mean NPIF was 85.60 ± 34.55 L/min, being 100.83 ± 36.42 L/min for men and 77.98 ± 31.13 L/ min for women (p = 0.001). In the mild AR group, NPIF was 107 ± 32.85 L/min, and in the moderate/severe group it, was 82.91 ± 33.97 L/min (p = 0.016). Considering the smoking habit, the NPIF was 89.71 ± 37.69 L/min for non-smokers, 89 ± 30.71 L/min for ex-smokers, and 68.88 ± 15.77 L/min for smokers (p = 0.034).

The NPIF in patients with a deficit in DAO activity was 76.30 ± 28.40 L/min compared to 93.62 ± 37.50 L/min in patients with normal enzymatic activity (p = 0.010). No linear correlation was found between DAO activity levels and NPIF in the whole sample (ρ = 0.04; p = 0.676) (Figure 1). In the group with mild AR, the NPIF was 81.25 ± 7.5 L/min for DAO deficiency and 120 ± 33.16 L/min for normal enzymatic activity (p = 0.031). In the group with moderate/severe AR severity, it was 75.86 ± 29.53 L/min for the deficit activity and 89.4 ± 36.71 L/min for normal activity (p = 0.057). The results are shown in Table 1.

Symptomatology

The typical symptoms of rhinitis and histamine intolerance were evaluated according to DAO activity (Table 2). A patient with DAO activity deficit has a higher risk of suffering from migraine at least twice a month

(OR = 2.89; p = 0.041), gastrointestinal discomfort (OR = 2.72; p = 0.012), and constipation/diarrhea without apparent cause (OR = 2.62; p = 0.016).

ESPRINT-15

The ESPRINT-15 questionnaire reached a mean score of 2.7 ± 1.21, being 2.5 ± 1.30 in men and 2.76 ± 1.15 in women (p = 0.319). Depending on the severity, patients with mild rhinitis had a score of 0.9 ± 0.3, and patients with moderate/severe rhinitis had a score of 2.8 ± 1 (p < 0.000). According to DAO activity, no significant differences were found (p = 0.815), with a score of 2.7 ± 1 in the activity deficit group and 2.6 ± 1.3 in the normal activity group. No linear correlation was found between the ESPRINT-15 score and the DAO activity levels (rho = –0.06; p = 0.543).

Discussion

AR is among the most common diseases worldwide and generally persists throughout life. It has been estimated that the prevalence of referred AR is ~ 2 – 25% in children and 1% to more than 40% in adults [1]. The mechanisms of the allergic reaction are being better understood over time, although histamine remains one of the main factors of the allergic reaction. A genetic or acquired error in the enzymatic catabolic function of DAO causes an imbalance of accumulated histamine and the capacity for its metabolization, and this is called histamine intolerance [2, 5, 6]. This relationship between the enzyme and the amount of extracellular histamine could play a role in the pathophysiology of the allergic diseases and therefore, in persistent AR. Although some studies measured DAO intracellularly or in serum,[19,26] this study did not measure the quantity but the activity of the enzyme. Therefore, this study was the first to analyze the possible relationship between an imbalance in the metabolism of histamine due to a DAO activity deficit and the symptoms of persistent AR.

There are few reports that have studied the relationship between AR and DAO deficit [17, 18, 19]. Some pointed to the possible use of DAO levels as an allergic biomarker in this type of patient [19], while others pointed out that there could be subgroups of AR patients with a different disease course due to single nucleotide polymorphisms (SNPs) that would cause DAO enzyme malfunction and an increased histamine accumulation even in situations where the amount of DAO is within normal parameters [6, 17, 18]. Specifically, the most relevant SNPs affecting DAO enzyme functionality in Caucasian individuals, like our population, are rs10156191, rs1049742, rs2268999, and especially rs1049793 [27]. This study is the first to measure the DAO enzymatic activity and to perform an objective measurement of the nasal flow using the NPIF in persistent AR.

The results show that about half of the patients with AR suffer from a deficit of DAO activity. These data are consistent with the high prevalence found in other pathologies [6, 13, 16]. Despite this, the results should be viewed with caution, requiring their validation in future control group studies. Likewise, the presence of other symptoms typically associated with histamine intolerance was evaluated, showing that the presentation of gastrointestinal symptoms and migraine is more frequent in patients with a deficiency of enzyme activity. These results are consistent with those observed in previous studies evaluating this type of pathology, in which a decrease in DAO activity was found in 80 – 90% of patients [6, 13, 16]. On the other hand, the presence of dermatological symptoms was not associated with a deficit in DAO activity, although the presence of both entities simultaneously does not seem to be so clear, being found only in small subgroups in the literature [11, 12, 28]. The low specificity and complex variability of symptoms undoubtedly contribute to the current difficulty in creating associations between symptoms and DAO deficit, as well as achieving consensus on the diagnostic criteria for histamine intolerance [6, 29, 30].

Patients with moderate/severe rhinitis had lower enzyme activity compared to patients with mild rhinitis. It seems logical that if a greater accumulation of histamine occurs, the severity of AR will be greater. This finding could be useful in future studies, making it possible to use the DAO activ-

ity levels as a biomarker to differentiate between severity groups in AR [19]. There is a higher degree of nasal obstruction measured objectively by NPIF in patients with DAO activity deficiency, regardless of the severity group of rhinitis. The lower the catabolic activity of the DAO enzyme, the lower the NPIF observed, but without a linear association between parameters. With these results, and as it was suggested in previous studies, the clinical course of AR might be altered in patients with impaired histamine metabolism [17]. There are many causes of malfunction or decrease in DAO activity [5, 11, 31, 32, 33] For example, carriers of DAO C2029G mutated allele tend to develop more severe symptoms of rhinitis and other histamine intolerance-related symptoms [17, 18, 34]. Therefore, in future studies this fact must be corroborated.

Despite this association, no significant differences or correlations were found in the quality of life measured with the ESPRINT-15 questionnaire between the groups with normal DAO activity and deficient DAO activity. This is in contrast to recently described findings in which linear correlations were obtained between AR severity and blood DAO levels [19]. Therefore, it is convenient to differentiate between the level of DAO in blood (previous studies) and its activity (current study). Our results indicate that variations in DAO function do not affect the quality of life of patients with AR despite the fact that they may be associated with a worsening of the NPIF.

Among the limitations of this study, it is important to highlight the impossibility of analyzing the real prevalence of DAO activity deficit, since patients were recruited solely from an outpatient department and the data collected could thus not be extrapolated to the general AR population. Subjects presenting only mild and intermittent symptoms may not recognize their condition as treatable, which could influence patient motivation to attend the consultation or accept being referred from primary care services. This may also apply to subjects presenting symptoms all year round and who may therefore consider their symptoms simply as a fact of life rather than a disease requiring treatment [35, 36]. This could be one of the reasons for the distribution of mild and moderate/severe AR found. Other limitations would be the cross-sectional design without a control group, the lack of other determinations (IgE, histamine, allergens), the lack of follow-up, or the lack of repeated DAO activity levels in the same patient [37, 38]. The strengths are the measurement of the NPIF and to relate it for the first time with DAO activity and histamine intolerance in AR, but even the analysis of DAO activity has been called into question as a diagnostic method for histamine intolerance [10, 25, 29, 30, 39]. This study opens up a range of possibilities, but the degree of the relationship found might not be enough to define the real link between DAO activity and AR severity.

On one hand, a cause-effect association between the severity of AR and the DAO activity cannot be concluded because AR is an entity in which many mediators are involved and possibly DAO is just one more actor in its pathophysiology. However, DAO activity could be considered in the future as a biomarker to differentiate between severity groups. On the other hand, there is some controversy about which measure is the best to assess DAO, whether its levels or its activity [19, 25, 29, 39, 40, 41, 42]. This work demonstrates that the same conclusions are not applicable for both parameters. Future studies in which the two parameters are evaluated together are necessary to discern the meaning and importance of each.

Finally, in recent years there is a tendency to relate the DAO activity deficit, and the consequent histamine intolerance, with a wide spectrum of signs and symptoms that can be improved with a low-histamine diet or enzyme supplementation therapy [14, 15, 31, 40, 43, 44, 45, 46]. Therefore, if the association between DAO activity deficiency and AR is confirmed, it could be considered as a therapeutic target for AR.

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Conflict of interest

There was no conflict of interest.

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Review

Histamine Intolerance Originates in the Gut

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Abstract: Histamine intolerance (HIT) is assumed to be due to a deficiency of the gastrointestinal (GI) enzyme diamine oxidase (DAO) and, therefore, the food component histamine not being degraded and/or absorbed properly within the GI tract. Involvement of the GI mucosa in various disorders and diseases, several with unknown origin, and the effects of some medications seem to reduce gastrointestinal DAO activity. HIT causes variable, functional, nonspecific, non-allergic GI and extra-intestinal complaints. Usually, evaluation for HIT is not included in differential diagnoses of patients with unexplained, functional GI complaints or in the here-listed disorders and diseases. The clinical diagnosis of HIT is challenging, and the thorough anamnesis of all HIT-linked complaints, using a standardized questionnaire, is the mainstay of HIT diagnosis. So far, DAO values in serum have not been established to correlate with DAO activity in the gut, but the diagnosis of HIT may be supported with determination of a low serum DAO value. A targeted dietary intervention, consisting of a histamine-reduced diet and/or supplementation with oral DAO capsules, is helpful to reduce HIT-related symptoms. This manuscript will present why histamine should also be taken into account in the differential diagnoses of patients with various diseases and disorders of unknown origin, but with association to functional gastrointestinal complaints. In this review, we discuss currently increasing evidence that HIT is primarily a gastrointestinal disorder and that it originates in the gut.

Keywords: histamine; diamine oxidase; gastrointestinal; biogenic amines; food intolerance; food malabsorption

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1. Introduction

Chronic and unexplained, functional, gastrointestinal (GI) symptoms impact more than 20% of the population. In histamine intolerance (HIT), the impairment of GI histamine degradation causes functional, nonspecific, non-allergic GI complaints and extra-intestinal complaints [1]. An unbalanced and elevated quantity of histamine in HIT seems to be the main consequence of the ingestion of histamine-containing foods [2]. Predominantly, in HIT, the intestinal enzyme diamine oxidase (DAO) has a reduced ability to metabolize and degrade histamine. Scientific evidence and studies to support this idea are increasing [3]. However, in association with various different disorders and diseases listed here, several with unknown underlying pathophysiologic mechanisms, the majority reports on additional unexplained, functional GI symptoms. Nonetheless, reliable standardized evaluations and/or laboratory tests for a definite diagnosis of HIT are still needed. HIT requires detailed anamnesis and diagnostic examination with available tests [4]. Taking into account the possible presence of additional GI diseases or disorders, the evaluation of all etiologic- and symptoms-causing factors is essential [5]. Subsequently, a personalized treatment with the targeted dietary intervention for each patient with HIT, using a histamine-reduced diet and/or oral supplementation with DAO, may help to provide sustained relief [6].

Here we review various disorders and diseases with unexplained, functional, GI complaints, and their parallels to HIT. We describe emerging evidence, and with help of

published studies, report on the link between reduced gastrointestinal DAO activity and HIT, and why this association demonstrates that HIT originates in the gut.

2. Histamine

Histamine [2-(4-imidazolyl)-ethylamine] is included in a group of biogenic amines with putrescine, cadaverine, and tyramine, amongst others, produced by bacterial fermentation [7]. The decarboxylation of the amino acid histidine results in histamine. Generally, histamine was discovered more than 100 years ago. The understanding of the central role of histamine in allergies and in immune regulations has led to the development of antihistamine medications [8].

A disproportionate amount of histamine in the body is suspected to result from the consumption of histamine-containing foods or drinks, and the reduced ability of enzymes to digest and degrade histamine. In foods, the manufacturing process, the cleanliness of materials, the microbial composition, and the fermentation influence the amount of histamine contained. The European Union allows the histamine content in food up to a maximum of 200 mg/kg in fresh fish and 400 mg/kg in seafood products [9]. Histamine contents of more than 40 mg per meal (0.75 mg/kg body weight) considerably increase the risk of scombroid poisoning [7]. However, this intoxication is in most cases caused by the consumption of decaying sea animals, which are contaminated with bacteria that cause high concentrations of histamine. Scombroid poisoning may also arise due to spoiled food because cooking, smoking, or freezing does influence [10], but not eliminate histamine. Usually, the consumption of low amounts of histamine and biogenic amines does not cause health problems in humans. Nonetheless, HIT is clearly separated from food allergies, like, e.g., peanut allergy [11], and is described as a non-allergic adverse reaction to ingested food [3].

3. Histamine Intolerance (HIT)

The term "histamine intolerance" is used similarly to lactose intolerance (LIT). LIT, with a deficiency of the enzyme lactase, shows parallels to the definition of HIT, with a deficiency of the GI enzyme diamine oxidase (DAO). Occasionally, HIT is also defined as "enteral histaminosis" or "sensitivity to dietary histamine" [2]. Although, HIT has also been suggested to be a metabolic disease [12], the inadequate digestion seems to cause excess histamine throughout the body that initiates a wide variety of symptoms. Nevertheless, histamine is also involved in the etiology of many common diseases [13].

Genetic expression of DAO is mainly in the small intestine, the ascending colon, the placenta, and the kidneys [14,15]. DAO activity, as shown in rats' intestines, increases from the duodenum to the ileum and is located in the intestinal villi [16]. It is synthesized by mature intestinal enterocytes and is constantly released from the intestinal mucosa into the gut as well as into the blood circulation, during digestion [17,18]. Ingested histamine, clearly below the dose of scombroid poisoning [19], is alleged to cause HIT-related symptoms. Human DAO and histamine N-methyl transferase, extra- and intracellular, respectively, are enzymes which catalyse the oxidative deamination of mono-, di-, and polyamines. Within the GI tract, DAO appears as the primary enzyme responsible for degrading ingested or microbiota-generated histamine [20,21].

The clinical diagnosis of HIT is still challenging, as standardized diagnostic tests are lacking. The thorough anamnesis of all HIT-linked complaints is the mainstay of HIT diagnosis. So far, a HIT questionnaire for volunteers, who randomly attended an outpatient clinic, has not proven useful for detection of HIT [22]. However, to evaluate patients with unexplained functional, nonspecific, non-allergic GI and extra-intestinal symptoms, suspected to have HIT, a questionnaire as shown in Table 1 may be helpful. We published a survey which listed 23 HIT symptoms in four categories including gastrointestinal, cardiovascular, respiratory, and skin complaints. This questionnaire contains questions about symptoms and the severity of them that patients experience due to ingestion of foods, and it is based on known symptoms related to the four histamine receptors [23]. In

this description the most common and most severe symptom indicated by HIT patients in more than 90% was bloating. Other very commonly related GI symptoms included: postprandial fullness and diarrhea (both in >70% of patients), abdominal pain (>65%), and constipation (55%) [1]. This questionnaire shown in Table 1, may help to clinically recognize and support the detection and diagnosis of HIT.

Table 1. Example of a standardized questionnaire for evaluation of patients with suspected histamine intolerance (HIT).

Severity of Complaints: No Symptoms (0), Mild (1) to Very Severe Complaints (5)						
			Gastrointestinal			
	0	1	2	3	4	5
Abdominal pain	o	o	o	o	o	o
Intestinal colics	o	o	o	o	o	o
Bloating	o	o	o	o	o	o
Diarrhea	o	o	o	o	o	o
Constipation	o	o	o	o	o	o
Nausea	o	o	o	o	o	o
Belching	o	o	o	o	o	o
Vomiting	o	o	o	o	o	o
Postprandial fullness	o	o	o	o	o	o
Menstrual cramps	o	o	o	o	o	o
			Skin			
	0	1	2	3	4	5
Pruritus	o	o	o	o	o	o
Eczema	o	o	o	o	o	o
Reddened skin	o	o	o	o	o	o
Swollen, reddened eye lids	o	o	o	o	o	o
			Cardiovascular			
	0	1	2	3	4	5
Headache	o	o	o	o	o	o
Dizziness	o	o	o	o	o	o
Hypotonia	o	o	o	o	o	o
Palpitations	o	o	o	o	o	o
Collapse	o	o	o	o	o	o
			Respiration			
	0	1	2	3	4	5
Rhinorrhea	o	o	o	o	o	o
Nose congestion	o	o	o	o	o	o
Sneezing	o	o	o	o	o	o
Asthma	o	o	o	o	o	o
	Additional Complaints (Please List Symptoms that Have Not Yet Been Listed)					
	0	1	2	3	4	5
	o	o	o	o	o	o
	o	o	o	o	o	o
	o	o	o	o	o	o

Please tick complaints which you experience mainly after ingestion of food. Adapted according to reference [1].

There are 50 known non-synonymous single-nucleotide polymorphisms for the gene which codes DAO [24] and the histamine receptors [25]. These seem associated with a manifold of clinical symptoms and hundreds of symptom combinations [1]. Nonetheless, this may help to explain the extensive individual variability of HIT-related GI and extra-intestinal symptoms. Furthermore, this genetic variety in HIT might influence disease expression and individual responses to diets or treatment. However, the functional and

clinical significance of these polymorphisms remains unknown. Another factor to consider is that histamine content in food is frequently unknown [26], and that it varies geographically depending on ripeness, storage time, and processing [6]. All of these variables need to be advised and may help to describe each person's unique and occasionally changing tolerance level.

However, a diagnosis of HIT may be supported with a low serum DAO value <10 U/mL (normal >10 U/mL) [27]. GI symptoms, indicative of HIT, and a reduction of these when following a histamine-reduced diet, help to support a successful diagnosis [3]. Although, DAO values in serum are not established to correlate with DAO activity in the gut, a significant increase in serum DAO due to a strict histamine-reduced diet was demonstrated in patients with HIT [28]. Subsequently, a correlation between low DAO values and symptoms of HIT, and a response to histamine-reduced diet and/or to oral diamine oxidase supplementation was shown [29,30]. Some findings suggested that serum DAO values agree with symptoms of HIT [31]. In vitro and in vivo observations in mice, including administration of DAO into the gut lumen, were found to be a valid treatment for histamine-induced intestinal dysfunctions [32]. DAO made from the white pea (*Lathyrus sativus*) prevented histamine toxicity in vitro in human epithelial colorectal adenocarcinoma cells [33].

Currently, serum DAO is being determined using a radio extraction assay. However, this analytical method has limitations because only a relative amount of DAO in serum is quantified. With this method, a human DAO standard is not commercially available and absolute DAO quantities cannot be measured. Therefore, the development of new assay methods have shown an enzyme-linked immunosorbent assay, using human DAO as a standard, to be more accurate [34]. Nonetheless, the search for HIT diagnostic tests continues.

4. Histamine Intolerance (HIT) Associated with Gastrointestinal (GI) Disorders

The various disorders and diseases, of which several are with unknown etiology, listed here are mainly presented with unexplained functional, nonspecific, non-allergic GI symptoms. Predominant symptoms are abdominal pain, bloating, and diarrhea. The GI symptoms include multiple individual combinations, which are also indicated by patients with HIT, with or without extra-intestinal symptoms.

However, DAO activity has been proposed as a marker of the integrity of intestinal mucosa. A recent study analyzed the molecular effects of histamine in human ileal neuroendocrine tumor cells, which are a model for gut enterochromaffin cells. The results indicated that enterochromaffin cells participate in intestinal intolerance or allergic reactions to food constituents associated with elevated histamine levels [35]. In inflammatory bowel diseases, reduced DAO activity was related to the degree of mucosal damage [36]. In Crohn's disease, DAO was discussed as a marker for disease assessment [37]. Moreover, histamine content and secretion were found to be significantly increased in affected mucosa in Crohn's disease and in ulcerative colitis [38]. Intestinal mucosa diamine oxidase activity was shown to reflect intestinal involvement in Crohn's disease. Additionally, histamine was concluded to contribute to the mucosal reactions in the intestine and to reflect the degree of colonic inflammation [39]. Measurement of gut DAO activity was stated as a biologic marker of colorectal proliferation [40] and histamine catabolism was reported to be lower in the colonic mucosa of patients with colonic adenoma [41]. According to reports, in oncologic patients receiving chemotherapy, DAO activity may be a predictor of intestinal mucosal damage [42]. Serum DAO activity was reported to be a predictor of GI toxicity and malnutrition due to anticancer drugs [43]. Some results in children support the hypothesis of DAO being a marker of small intestinal functional integrity [44].

Histamine and DAO measurements, in vivo and in vitro, beyond any doubt need further investigation to shed light on the changes in gut tissues during various diseases and disorders.

4.1. Irritable Bowel Syndrome (IBS)-Like Disorders

Functional dyspepsia (FD), IBS, and small intestinal bacterial overgrowth (SIBO) are commonly reported, but solely symptom-oriented conditions. These clinical syndromes continue to be imprecise and were therefore re-named to "IBS-like" disorders [45]. They have no established pathophysiology, however, emerging evidence suggests that this paradigm may need revision [46]. In general, there is a lack of specificity of symptoms. Symptoms alone or symptom complexes can rarely, if ever, be used diagnostically. However, 80% of IBS patients identified food, including histamine, to be triggers for their GI symptoms [47]. Currently, IBS is defined using—and discussed within—the Rome IV criteria [48]. Furthermore, histamine was recently named to act as a key mediator in IBS [46]. FD is with absence of an organic disease a disorder with heterogeneous symptoms in the epigastric region. Patients with HIT report abdominal pain and postprandial fullness to be prominent symptoms and these are described in FD also as main symptoms. SIBO occurs with an abnormal increase in the overall bacterial population in the small intestine. Bloating and abdominal pain are primary symptoms in SIBO, very much comparable to HIT complaints [1]. However, there is an imprecise clinical overlap between IBS, FD, SIBO, and IBS-like disorders [49], and it is suspected that a number of different pathogenic mechanisms may be responsible. Dietary nickel (Ni) was described as a possible etiology for GI complaints in patients with IBS-like symptoms [50]. Remarkably, several foods described as containing Ni are also not well digested by HIT patients because of their high histamine content.

Since there are symptom correlations in FD, IBS, and SIBO with HIT, determination of serum DAO values in well-defined patients may help to elucidate this link.

4.2. Non-Celiac Gluten Sensitivity (NCGS)

Due to all of these disorders being increasingly discussed in the media, a growing number of people are changing their diets. Currently, approximately 20% of the population are avoiding gluten in food because of a self-diagnosed, new clinical condition, referred to as non-celiac gluten sensitivity (NCGS). Unproven hypotheses of supposed health benefits due to gluten-free eating are wide-spread [51]. There are no medical tests available for the diagnosis of NCGS, and patients were also named "people without celiac disease avoiding gluten" [52]. Mainly, they are self-diagnosed and perform self-treatment with a gluten-free diet. It has been proposed that NCGS patients are a certain group of patients with IBS. Recently, a newly created term "non-celiac wheat sensitivity" has been suggested as a more accurate definition [53]. Moreover, widely used gluten-containing bakery goods and beers are prepared with histamine-producing yeast [54,55]. Additionally, foods like pasta, pizza, and bulgur are very commonly consumed with histamine-containing tomatoes [56] and seasonings. Generally, several gluten-free foods are low in histamine [57] and an increasing number of gluten-free products, including breads, are already labeled gluten-free and yeast-leavened [58]. Furthermore, we reported that in NCGS the GI and extra-intestinal symptoms resemble those found in HIT [59]. However, the reduction of gluten-containing food and drinks cuts the quantity of parallel consumed histamine. This may help to explain the current extraordinary popularity of gluten-free food. Lastly, the pathophysiology of these symptom-oriented disorders are unknown and HIT, with its plethora of symptoms, may play a role.

4.3. Food Intolerance/Malabsorption

There is growing public interest in adverse reactions to food that may influence and impair digestion. Food intolerance/malabsorption and people who experience GI reactions to food are reported to affect 20% of populations. Particularly the sugars (lactose and fructose), proteins (gluten), and biogenic amines (including histamine) are widely consumed and trigger GI reactions to food. However, quantity, type, and composition of dietary carbohydrates and proteins influence digestion and the metabolic output of gut

microbiota [60]. In patients with HIT, a deranged gut flora and a change of the intestinal bacterial composition was demonstrated [61].

One or a combination of various food components cannot be degraded and/or absorbed properly within the gut. Combinations of LIT and fructose malabsorption were reported in >30% of patients with a carbohydrate intolerance/malabsorption [62]. In lactose-intolerant patients, the effect of HIT with different perceptions of functional, non-specific, non-allergic GI symptoms was described [63]. During H_2 breath tests of patients with LIT, the presence of additional fructose malabsorption and HIT significantly increased expiratory H_2 values. This indicated that HIT embodies a separate GI disorder as food intolerance/malabsorption [64]. We described the fact that 55% of the patients with carbohydrate intolerance/malabsorption have serum DAO values below 10 U/mL as an indicator for HIT [5]. Further studies and descriptions of food intolerance/malabsorption, including the changes of the microbiota, in various combinations of intolerance/malabsorption, including HIT, are certainly needed.

4.4. Medications

Due to the increased release of histamine, or an inhibition of DAO, a variety of medications may influence HIT-related complaints or induce HIT [1,65,66] (Table 2). Approximately 20% of Europeans use DAO-inhibiting drugs on a regular basis, which significantly increases their susceptibility to adverse effects of ingested histamine [67]. Several of these drugs, including high-dose acetylsalicylic acid and nonsteroidal anti-inflammatory drugs, are available over the counter. However, they may cause GI side effects, including, with prolonged and high-dose use, an increased risk for GI bleedings [68]. Particularly, when exploring HIT-related symptoms, long-term treatments with these drugs need assessment, as well as reflection concerning the estimation of serum DAO values.

Table 2. Medications which may influence diamine oxidase and/or histamine.

Medications	Generic Name
Analgesics	Acetylsalicylic acid, Metamizole, Morphines, Nonsteroidal anti-inflammatory drugs, Pethidine
Antiarrhythmics	Propafenon
Antibiotics	Cefuroxime, Cefotiam, Isoniazid, Pentamidine, Clavulanic acid, Chloroquine
Antidepressants	Amitriptylline
Antifungal	Pentamidine
Antihypertensives	Verapamil, Alprenolol, Dihydralazine
Antihypotensives	Dobutamine
Antimalarial	Chloroquine
Broncholytics	Aminophylline
Cytostatics	Cyclophosphamide
Diuretics	Amiloride
H2 receptor antagonists	Cimetidine
Local anesthetics	Prilocaine
Motility agents	Metoclopramide
Mucolytics	Acetylcysteine, Ambroxol
Muscle relaxants	Pancuronium, Alcuronium, D-Tubocurarin
Narcotics	Thiopental
Radiological contrast media	
Vitamines	Ascorbic acid, Thiamine

Adapted according to references [2,62].

4.5. Disorders Associated with Mast Cells

Mast cells are essentially involved in immunity and inflammation. Endogenous histamine is stored mainly in mast cells and basophils. The mast cells' histamine release appears linked to GI-involving diseases like celiac disease (CD), eosinophilic gastroenteritis (EGE), and mast cell activation syndrome (MCAS) [4].

CD is a well-defined autoimmune disorder characterized by a gastrointestinal mucosal reaction to ingested gluten proteins. An overlap between CD and irritable bowel syndrome (IBS) exists and an improvement of symptoms with gluten restrictions in IBS was reported [69]. Mast cells releasing histamine and other inflammatory mediators have been linked with CD, showing that mast cells infiltrating the mucosa were associated with the severity of mucosal damage [70]. HIT was found in 55% of non-responsive CD patients and seems to play an important role in CD [71]. Originally, the Mas-related G protein-coupled receptor family (Mrgprs) members were identified as itch receptors in cutaneous sensory neurons. Now, they have become a target in abdominal pain research. Recently, a function for Mrgprs in the mouse gut nociceptive innervation and through Mrgprs elevation of histamine was found [72]. Given these functions of Mrgprs in humans, these findings deserve future research and may help to elucidate pathophysiology of HIT.

The underlying mechanisms of EGE are remain unknown. EGE is characterized by eosinophilic infiltration into the gastrointestinal mucosa. With the eosinophil–mast cell axis, which is involved in functional GI disorders, mast cells induce eosinophils and eosinophils activate mast cells in a co-dependent manner. Several symptoms in EGE, including GI complaints [73] resemble complaints in HIT [1]. It was suggested that cytokines combined with proliferation of eosinophils and histamine-releasing mast cells are involved in EGE [74]. The influence of a histamine-reduced diet on gastrointestinal symptoms in EGE remains to be discovered.

Mast cell activation disorders have a certain relation of GI and extra-intestinal complaints with HIT. It was questioned whether HIT patients, some or all, could be seen within the mast cell activation disorders spectrum [75]. However, HIT mainly causes GI symptoms. A coexistence of the hypermobility spectrum disorder, Ehlers-Danlos syndrome, and the postural tachycardia syndrome exists [76]. Individuals with these syndromes have frequently functional GI disorders fulfilling criteria according to Rome IV [77] and present complex clinical challenges. The postural orthostatic tachycardia syndrome is associated with increased occurrence of IBS-like symptoms and is described according to abnormal increase in heart rate occurring after sitting or standing up. Future studies need to elucidate the pathophysiology of hypermobility spectrum disorders and postural orthostatic tachycardia, including evaluation of HIT in these syndromes. The role of histamine and a therapeutic effect of a histamine-reduced diet, and the effect of oral DAO capsules, remain to be determined.

4.6. Helicobacter Pylori (H.p.) Infection

In patients with unexplained functional GI symptoms, an *H.p.* infection needs to be considered. Accordingly, if it is detected, an eradication therapy is required [78]. Gastrin and histamine are the main stimulants of gastric acid secretion, and they, combined with *H.p.*, influence acid secretion [79]. Although the involvement of *H.p.* in functional dyspepsia is controversial, some studies from areas with a high prevalence of *H.p.* reported that eradication improves dyspepsia. Some association of *H.p.* infection and CD was reported because CD patients had significantly lower rates of gastric *H.p.* infection [80]. This argues that evaluations of GI infection with *H.p.*, the most prevalent human pathogen, with HIT are also needed.

5. Histamine Intolerance (HIT) Associated with Other Disorders

In various disorders and diseases, of which several are of unknown etiology, no clear link from headache and urticaria complaints to dyspepsia can be established. However, several patients in evaluations of described disorders were reported with unexplained functional, nonspecific, non-allergic GI symptoms.

5.1. Headache

A specific pathophysiologic mechanism of headache is still unknown and migraines are defined as an untreatable disease [81]. Biogenic amines in wine, including histamine,

tyramine, and putrescine [82], have relevance for headache and histamine-related symptoms [83]. Red wines contain clearly more than double the histamine concentrations with >2200 µg/L, compared to white wines (~900 µg/L histamine) [6]. An increased risk for migraines was demonstrated in patients with some DAO genotypes and allelic variants [84]. A high incidence of DAO deficiency at nearly 90% was observed in migraine patients [85]. Recently, a study demonstrated that oral ingestion of capsules with DAO significantly reduces headaches in migraine patients [86]. Subsequently it was shown that headaches in HIT patients—as one of the many symptoms in HIT—were considerably reduced due to oral DAO supplementation [30]. The organic wine industry was reported to lower the amount of histamine in wine. This decreases headaches and other adverse effects usually provoked by drinking wine [87]. Associations between migraine, celiac disease, non-celiac gluten sensitivity, and low activity of DAO were hypothesized. It was stated that patients with low serum DAO values were more severely impacted by migraine than healthy persons with normal DAO activity [88]. Bloating with headache was mentioned by 63% of HIT patients as one of the most common HIT-related symptom combinations [1]. Thus, the histamine content of consumed food may play a key role in triggering migraines and headaches, and there are certainly additional evaluations necessary.

5.2. Urticaria

Urticaria is a relapsing-remitting skin disease with unknown etiology. The majority of cases are called idiopathic or autoreactive. It has a significant impact on affected patients and reduces their quality of life. The mainstay of medical therapy is symptom management, including the use of H1 antihistamines. Histamine-releasing mast cells and basophils are known as primary inflammatory cells in urticaria. Degranulation and release of vasoactive substances, including histamine and other pro-inflammatory mediators, causes vasodilation, sensory nerve activation, plasma extravasation, dermal edema, and wheals. Although further evaluations are needed, it appears that some patients have dietary triggers contributing to this skin disease and a number of food ingredients have been reported to worsen symptoms [89]. In patients suffering from urticaria, accompanied by functional GI symptoms, a histamine-reduced diet was demonstrated therapeutically useful, simple, and economically efficient. The diet decreased symptoms and increased quality of life in urticaria patients [90]. Additionally, oral DAO supplementation was found to improve the degree of urticarial activity score, which was inversely correlated with the levels of serum DAO [91]. Dietary intervention in urticaria patients with histamine-reduced diet and/or oral DAO capsules obviously needs additional investigations.

5.3. Further Diseases and Disorders

Occurrence of HIT, causing GI complaints, was described in some patients with rare diseases, such as primary epiploic appendagitis, beta-thalassemias minor, and Gullo syndrome [92]. However, it has also has been reported in several other cases and further groups of patients with HIT-related symptoms. In all of these examples, the reduction of histamine in food with initiation of a histamine-reduced diet was helpful to decrease complaints.

Recently, a patient with already performed Nissen fundoplication had recurring gastroesophageal reflux symptoms with coughing and increased throat clearing. Lung function and gastroesophageal reflux disease were tested but not found as the cause of these symptoms. After a successful diagnosis of HIT, a histamine-reduced diet resulted in apparent improvement of the patient's complaints [93].

In a female patient with anorexia nervosa, HIT-related symptoms arose. After she followed a histamine-reduced diet, she experienced weight gain and improvement of GI symptoms [94].

Drinking wine with high histamine content caused coughing and wheezing with bronchoconstriction in patients with HIT [95].

Etiology and triggers of atopic dermatitis are still under debate. The treatment of atopic dermatitis, in a patient who also had HIT-related GI symptoms, was successful with a histamine-reduced diet [96].

Interestingly, histamine secretion from bacteria within the gut of mice was shown to have immunological consequences within the lung [97]. On the other hand, a pilot study reported on a histamine-reduced diet, which might have had an active and direct impact on asthma symptoms in children, without known HIT [98].

Somewhat conflicting results are reported on DAO and histamine values in patients with multiple sclerosis (MS), an autoimmune disease of the central nervous system. Incompatible low DAO and low histamine serum levels have been documented in MS [99]. However, the pathogenesis and an association to HIT are unknown, but this again documents the need for further investigations.

Usually, GI endoscopy with histologic evaluation of GI mucosa and radiological evaluation, including ultrasound, of the abdomen are valuable additional options for examination of patients, especially aged >55 years, with functional, nonspecific, non-allergic GI symptoms [100]. If signs of GI infection are evident, then specific anti-microbial or anti-parasitic treatments, that may reverse the disease, need consideration.

6. Discussion

Generally, the growing number of scientific studies has led to a better understanding of the role of food ingredients causing GI complaints. If biogenic amines, including histamine, cannot be absorbed and digested properly in HIT then this causes non-allergic, functional, nonspecific GI and extra-intestinal complaints. GI bacteria use catabolic enzymes to degrade and ferment carbohydrates and proteins from ingested food [101], as well as in HIT, a changed intestinal bacterial composition was reported.

Although there is limited availability of sufficiently sensitive and specific diagnostic procedures, it seems essential to assess HIT individually with the currently available methods. The thorough anamnesis of all HIT-linked complaints is the mainstay of HIT diagnosis. However, for evaluation of patients with symptoms, suspected to have HIT, the questionnaire indicated here (Table 1) may be helpful. Medical personnel should be aware that a large number of HIT patients have, to a certain extent, GI complaints. However, distinct patients may present only single symptoms like, e.g., postprandial vertigo or headache. Additionally, several studies demonstrate the growing importance of serum DAO determinations. So far, some studies conducted report on an insufficient low number of patients. This demonstrates the need for further evaluations of HIT in clearly defined and larger patient groups.

After detailed diagnosis of HIT, the GI complaints and extra-intestinal symptoms can be decreased by reducing the consumption of histamine and/or oral DAO supplementation. Capsules containing DAO seem helpful but there is an ongoing discussion about the therapeutically helpful dosage [102]. Each patient's tolerance level should also be considered, when recommending dietary restrictions for long-term symptom reduction. Nonetheless, the permanent compliance with a locally-available, histamine-reduced diet, is challenging for patients. Certainly, this represents the future need of the histamine content indication on food labels. Improvements and developments of new methods for the determination of histamine and biogenic amines in food will allow this [103–105].

A strong association of histamine and HIT with functional GI complaints in various disorders and diseases is assumed, several of these with a so-far unknown origin. Therapeutically helpful, in many ways, is the reduction of the food histamine load. However, if HIT is present, an experienced, a registered dietician may help to design an individually tailored diet. The reduction of ingested histamine with a histamine-reduced diet is helpful for HIT-related symptoms and available at low cost. This seems to positively influence the pathophysiologic process, including inflammation, and accomplish symptom reduction. The oral supplementation with DAO capsules represents an additional therapeutic option to lower HIT-related symptoms. So far, an agreement for dosing of oral DAO supplements

has not been established. Commercially available capsules contain 4.2 mg extracted pig kidney proteins with 0.3 mg DAO. These are suggested up to three times per day before meals. Although, it was reported that a higher activity of DAO seems required for a satisfactory histamine degradation [102]. The dietary intervention with histamine-reduced diet and/or oral supplementation of DAO clearly needs additional investigations. Interdisciplinary management is necessary, so that all etiologic and therapeutic possibilities are included in the evaluation of patients with HIT [4,106]. The diagnosis of HIT helps patients to put their symptoms into context, to reduce complaints and to improve their quality of life. This shows why additional scientific evaluations in these demonstrated diseases and disorders are certainly necessary. Nonetheless, the development of new laboratory methods will improve determination of DAO and histamine in serum, and histamine in food.

7. Conclusions

In conclusion, HIT seems to play a more significant role in GI disorders and complaints, and in several extra-intestinal disorders, than so far anticipated. Overall, scientific evidence of histamine involvement and HIT in the demonstrated diseases and disorders is scarce but increasing. A detailed diagnosis of HIT, with currently available methods and tests, including the questionnaire (Table 2), is helpful and necessary to evaluate each patient with HIT-related GI and extra-intestinal complaints. With the increasing scientific evidence, we demonstrate, that HIT is primarily a GI disorder and that it originates in the gut.

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Abbreviations

CD	Celiac Disease
DAO	Diamine oxidase
EGE	Eosinophilic gastroenteritis
FD	Functional dyspepsia
GI	Gastrointestinal
H.p.	*Helicobacter pylori*
HIT	Histamine intolerance
IBS	Irritable bowel syndrome
LIT	Lactose intolerance
MCAS	Mast cell activation syndrome; Mrgprs, Mas-related G protein-coupled receptor family
NCGS	Non-celiac gluten sensitivity
SIBO	Small intestinal bacterial overgrowth

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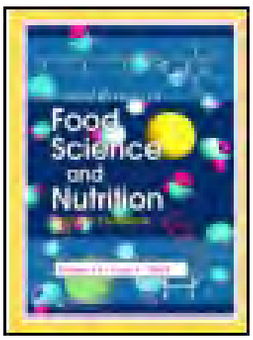

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REVIEW

Considering histamine in functional gastrointestinal disorders

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ABSTRACT

In westernized countries, adverse reactions to ingested foods are reported to affect up to 20% of the population. Functional, nonspecific, non-allergic gastrointestinal complaints are mainly due to the intolerance/malabsorption of carbohydrates (lactose and fructose), proteins (gluten), and biogenic amines (histamine). Food intolerance/malabsorption is defined by one or several of the above mentioned food components not being degraded and/or absorbed properly within the gastrointestinal tract. Food intolerance/malabsorption causes variable, functional, nonspecific, non-allergic gastrointestinal and extra-intestinal complaints, and a detailed diagnostic workup for all possible etiologic factors in individual patients is essential. Usually, evaluation for histamine intolerance is not included in differential diagnoses of patients with functional, nonspecific, non-allergic gastrointestinal complaints. A targeted dietary intervention for single or possibly combined intolerance/malabsorption is required. In this article, we review currently discussed differential diagnoses and available tests for intolerance/malabsorption. Accordingly, we aim to outline why including histamine and, histamine intolerance, should be considered in differential diagnoses of patients with functional, nonspecific, non-allergic gastrointestinal complaints.

KEYWORDS

Histamine; lactose; fructose; diamine oxidase; food intolerance; food malabsorption; mastozytosis

Introduction

Adverse reactions to ingested foods causing functional, nonspecific, non-allergic gastrointestinal complaints are due to the intolerance/malabsorption of carbohydrates (lactose and fructose), proteins (gluten), and biogenic amines (histamine). However, there is a lack of understanding of these functional, nonspecific, non-allergic symptoms and diagnoses are limited by the lack of accepted standardized tests for the underlying mechanisms (Talley 2020). Due to these disorders being increasingly discussed in the media, a growing number of people change their diets. Nevertheless, intolerance/malabsorption is causing variable gastrointestinal (GI) and extra-intestinal complaints (Mitchell et al. 2019). Generally, symptoms are observed subjectively, and intolerance/malabsorption-caused symptoms are not dependably confirmed by medical tests. Scientific evidence to support this postulated link is increasing, but reliable comprehensive evaluations and/or laboratory tests for definite diagnoses are still needed (Reese et al. 2017).

Food intolerance/malabsorption requires detailed diagnostic examination with available tests for all possible etiologic aspects of each individual patient and, subsequently, personalized treatment with individual dietary plans. Only the targeted dietary intervention for each single, or possibly combined, intolerance/malabsorption may help to provide sustained relief (Enko et al. 2016). Here we review currently discussed differential diagnoses and available tests, and outline why including histamine intolerance (HIT) in the differential diagnoses of intolerance/malabsorption should be considered for patients with functional, nonspecific, non-allergic GI and extra-intestinal complaints.

Food intolerance and malabsorption

There is growing public interest in food intolerance/malabsorption for people who experience reactions to food. Food intolerance/malabsorption causes functional, nonspecific, non-allergic GI complaints and extra-intestinal symptoms. In westernized countries, adverse reactions that may influence and impair digestion are reported to affect up to 20% of the population (Mitchell et al. 2019).

Recent investigations have led to an improved understanding of food components, particularly of sugars (lactose and fructose), proteins (gluten), and biogenic amines (including histamine). In most cases of intolerance/malabsorption one or a combination of the above described food components cannot be degraded and/or absorbed properly within the GI tract. GI bacteria then use various catabolic enzymes to degrade and ferment ingested food. However, experiments suggest that the quantity, type and composition of dietary carbohydrates and proteins change the metabolic output of these microbes (Schink et al. 2018; Albenberg and Wu 2014).

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Carbohydrate intolerance/malabsorption

Carbohydrate intolerance/malabsorption is mainly caused by lactose intolerance and fructose malabsorption. Lactose intolerance, with an estimated global prevalence of approximately 70 percent in the world's population, is present in most countries. It is the inability to digest the disaccharide lactose which is mainly found in milk and dairy products. Lactose malabsorption is due to the deficiency of the lactose-degrading enzyme lactase, which hydrolyzes lactose into two monosaccharides, namely glucose and galactose (Storhaug, Fosse, and Fadnes 2017; Ugidos-Rodríguez, Matallana-González, and Sánchez-Mata 2018). If the ingestion of lactose is accompanied by GI symptoms, then it is referred to as lactose intolerance. The clinical diagnosis of lactose intolerance is mainly performed with a hydrogen breath test. During this test a drink containing 50 g of lactose is ingested and the exhalation of hydrogen (H_2) is measured subsequently. Genetic testing for lactase persistence may be performed, but the clinical use of genetic tests has been criticized. It does not describe clinical manifestations or symptoms, and it is limited due to an increasing number of known single nucleotide polymorphism mutations (Casellas et al. 2010).

As outlined below, the diagnosis of histamine intolerance (HIT) may be supported by measurements of a serum diamine oxidase (DAO) value. During H_2 breath tests, performed in patients with lactose intolerance and serum DAO values $<$ 10 U/mL, higher end-expiratory H_2 levels were observed. In addition, these patients reported more symptoms during the H_2 breath tests when compared to lactose-intolerant patients with DAO values $>$ 10 U/mL. This study described the effect of HIT on the perception of functional, nonspecific, non-allergic GI symptoms and concludes that HIT needs to be considered in lactose-intolerant patients (Enko et al. 2017).

The growing amount of ingested fructose, particularly high-fructose corn sirup, is resulting in an increasing occurrence of complaints caused by fructose malabsorption. In this malabsorption the monosaccharide fructose is not adequately absorbed by the epithelial glucose transporters, GLUT-5 and GLUT-2, in the intestinal mucosa (Gibson et al. 2007). Fructose malabsorption is described in several studies and detailed reviews (Latulippe and Skoog 2011; Tuck et al. 2017).

With intolerance/malabsorption, the sugars lactose and fructose reach the large intestine where they are a bacterial substrate. This results in fermentation with hydrogen production. Therefore, the clinical diagnosis of fructose malabsorption is also performed with the H_2 breath test. Usually, the breath test for diagnosis of fructose malabsorption is performed with a drink containing 25 g fructose. There is a dose-dependent and limited absorption capacity with 50 g fructose described in up to 80% of healthy individuals (Jones, Butler, and Brooks 2011).

Evaluations have reported combined lactose intolerance and fructose malabsorption in more than 30% of patients with carbohydrate intolerance/malabsorption and functional, nonspecific, non-allergic GI disorders (Wilder-Smith et al. 2013). We described a high prevalence of 55% serum DAO values $<$10 U/mL, indicative of HIT, occurring in patients with carbohydrate intolerance/malabsorption. Seven various combinations of intolerance/malabsorption were described in patients with functional, nonspecific, non-allergic GI symptoms (Enko et al. 2016). Further descriptions and studies on symptom perception and clinical differences also the changes of the microbiome, in these various combinations of intolerance/malabsorption, including HIT, are needed.

Irritable bowel syndrome-like disorders

There is an imprecise clinical overlap between irritable bowel syndrome (IBS) and other IBS-like disorders. The most commonly reported symptom-oriented conditions are IBS, functional dyspepsia (FD) and small intestinal bacterial overgrowth (SIBO). All of these present with various functional, nonspecific, non-allergic GI symptoms, predominantly abdominal pain, bloating and diarrhea (Borghini et al. 2017). These GI symptoms, including multiple combinations of these, are also reported by patients with HIT (Schnedl, Schenk, et al. 2019).

Generally, there is a lack of specificity of symptoms, therefore symptoms alone or symptom complexes are hardly, if ever, diagnostic (Verdu, Armstrong, and Murray 2009). It is suspected that various pathogenetic mechanisms may be responsible for IBS (Chey 2016). However, 80% of IBS patients identified food, including histamine, as a possible trigger for their symptoms (Böhn et al. 2013). Currently IBS is defined and discussed within the Rome IV criteria (Schmulson and Drossman 2017). These clinical conditions, such as IBS, FD and SIBO continue to be nebulous and were therefore recently re-named IBS-like disorders (Borghini et al. 2017). Finally, the triggers of these symptom-focused disorders are not well understood and HIT, with its plethora of symptoms, may play a role.

In the search for etiologic factors, dietary nickel (Ni) was recently described as a possible causative factor in patients with IBS-like symptoms (Borghini et al. 2016). Interestingly, several foods described as containing Ni, including tomatoes, cocoa, mushrooms, shellfish, nuts, and canned foods contain histamine, and are consequently not well digested by HIT patients.

Celiac disease

Celiac disease (CD) or gluten malabsorption is a well-defined autoimmune disorder characterized by a reaction to ingested gluten proteins that occurs in genetically predisposed persons. Gluten proteins are mainly found in wheat, rye and barley. The diagnosis of celiac disease is performed with serologic testing and needs histologic confirmation in duodenal biopsies. Although there are a number of undetected "silent" celiac patients suspected, the frequency of biopsy-proven CD was found to be approximately 1% in the general population (Mustalahti et al. 2010). In celiac disease the gluten-free diet is the only effective treatment (Valenti et al. 2017). Patients with CD who do not comply

with a gluten-free diet are at risk for concomitant diseases including osteoporosis, lymphoma, and autoimmune disorders (Ventura, Magazzù, and Greco 1999). Histamine- and other inflammatory mediators releasing mast cells have also been linked with CD, showing that mast cells infiltrating the mucosa were associated with the severity of mucosal damage (Frossi et al. 2017).

Non-celiac gluten sensitivity (NCGS)

The range of gluten-related disorders has expanded with the description of a new clinical condition, non-celiac gluten sensitivity (NCGS) (Dale, Biesiekierski, and Lied 2019). Typically, patients with NCGS test negative with all available diagnostic tests for celiac disease (CD). However, since there are no diagnostic criteria available for NCGS, affected people were also named "people without celiac disease avoiding gluten" (Choung et al. 2017). Currently, up to 20% of the westernized world's population are avoiding gluten because of self-diagnosed NCGS with GI and/or extra-intestinal symptoms. Unproven hypotheses of supposed health benefits due to gluten-free eating are being spread (Vici et al. 2016).

A recently created term, non-celiac wheat sensitivity, has been proposed as more accurate. It allows for the inclusion of other suspicious symptom-causing non-gluten wheat components such as fructane, galactane, and amylase–trypsin inhibitors (Zevallos et al. 2017). In addition, it has been proposed that people with NCGS constitute a group of patients with irritable bowel syndrome (IBS), who are self-diagnosed and perform self-treatment by adhering to a gluten-free diet (Catassi et al. 2017; De Giorgio, Volta, and Gibson 2016).

Gluten-containing bakery goods and beers are frequently made with yeast, which produces histamine (Qi et al. 2014; Verheyen et al. 2015). Universally consumed food including pasta, pizza and bulgur are commonly consumed with tomatoes and seasonings which, because of their histamine content (Bolygo et al. 2000), are not digested well in people with HIT. Moreover, we reported that in NCGS the GI and extra-intestinal symptoms resemble those found in HIT. However, the reduction of gluten-containing food and drinks cuts the quantity of parallel histamine consumption (Schnedl et al. 2018). This may help explain the current extraordinary popularity of gluten-free food intake.

Helicobacter pylori (H.p.)

H.p. causing a change of the gastric environment and with this affecting the absorption of certain nutrients has been described (Franceschi et al. 2014). Although the involvement of an infection with *H.p.* in functional dyspepsia is controversial, some studies support the connection of *H.p.*-induced gastritis in the pathogenesis of dyspeptic symptoms. Recently, in areas with a high prevalence of *H.p.*, it was reported that *H.p.* eradication improves dyspepsia. Additionally, conflicting associations of *H.p.* with gastroesophageal reflux disease were published (Jonaitis, Pellicano, and Kupcinskas 2018). An association between *H.p.* infection and CD was demonstrated because CD patients had significantly lower rates of gastric *H.p.* compared to healthy patients (Lebwohl et al. 2013). In patients with functional, nonspecific, non-allergic GI symptoms, an *H.p.* infection needs to be considered and eradication therapy is required (Tomita, Oshima, and Miwa 2018). Evaluations of HIT in *H.p.* infection are needed because the main stimulants of gastric acid secretion are gastrin and histamine, which in concert with *H.p.* may increase or decrease acid secretion (Schubert 2017).

Generally, GI endoscopy with histologic evaluation of GI mucosa and ultrasound of the abdomen are valuable extra options for the evaluation of patients, especially aged >50 years with functional, nonspecific, non-allergic GI symptoms (Bai et al. 2018). If signs of infection are evident, then GI infections need consideration to start specific antimicrobial or anti-parasitic treatments that may reverse disease.

Mast cell activation syndrome (MCAS)

Intra-corporeal produced histamine appears to have less influence on HIT except in cases of mastocytosis. Mast cells (MCs) are heterogeneous, multifunctional cells basically involved in immunity and inflammation. Blood MCs produce various inflammatory and vasoactive mediators, and store them in intracellular, metachromatic granules (Varricchi et al. 2019). MCAS is a condition where an increased number of mast cells, inadequately, due to still unknown factors, release histamine and other mediators, resulting in a variety of symptoms. Generally, symptoms in MCAS are nonspecific and include: urticaria, flushing, abdominal cramps, diarrhea, collapse, and tachycardia. These may range from mild to severe, potentially life-threatening, especially in patients with mastocytosis and concomitant allergy. Recently, consensus criteria were defined for MCAS with severe, recurrent symptoms that, affect more than one organ, frequently in combination with hypotension and anaphylaxis. However, there certainly is a relation of functional, nonspecific, non-allergic GI and extra-intestinal complaints in MCAS and HIT. Nevertheless, HIT predominantly causes GI symptoms (Schnedl, Schenk, et al. 2019; Valent et al. 2019). Helpful for diagnosis in MCAS patients are serum tryptase values and, it was reported that DAO and tryptase significantly increase during anaphylaxis in MCAS patients (Boehm et al. 2019).

The Mas-related G protein-coupled receptor family (Mrgprs) members were identified as itch receptors in cutaneous sensory neurons. Now, they have become a target in abdominal pain research. Recently, a novel function for Mrgprc11 in the mouse gut nociceptive innervation was found (Van Remoortel et al. 2019) and in mice phenothiazine antipsychotics were shown to increase histamine levels over Mrgprb2 (Hou et al. 2019). Given these functions of Mrgprs in humans, the mentioned findings therefore deserve future research and may also help to elucidate pathology of HIT.

Eosinophilic gastroenteritis (EGE)

EGE is a digestive disorder that is characterized by eosinophilic infiltration into the gastrointestinal mucosa. The underlying mechanisms to this disease are still unknown. However, eosinophils and mast-cells recruitment and activation seem codependent. Mast cells induce eosinophils in the mucosa, and eosinophils in turn can activate mast cells. This is commonly accepted as the eosinophil–mast cell axis, involved in functional GI disorders, associated with increased visceral sensitivity and disordered motility. Although, in EGE corticosteroids seem the mainstay of effective therapies, alternative therapeutic regimens include mast-cell stabilizers and antihistamines. Symptoms in EGE like abdominal pain, nausea, vomiting, dyspepsia, diarrhea, eczema and rhinitis (Sunkara et al. 2019), resemble several complaints of HIT (Schnedl, Schenk, et al. 2019).

An own entity seems eosinophilic esophagitis as a chronic immune disease, characterized by a dense eosinophilic infiltrate in the mucosa of the esophagus. Food impaction and reflux-like symptoms are main complaints. The pathophysiology of this localized inflammation is also unknown, but it was suggested that several cytokines and, besides others, a proliferation of eosinophils and histamine-releasing mast cells is involved (D'Alessandro et al. 2015). It remains to be evaluated how a histamine-reduced diet influences MCAS and EGE.

Histamine

Histamine [2-(4-imidazolyl)-ethylamine] was discovered more than 100 years ago and it plays an important role in allergic reactions and inflammation (Jones and Kearns 2011). Histamine belongs to a group of biogenic amines including putrescine, cadaverine and agmatine, which are produced by bacterial decarboxylation of foods (Doeun, Davaatseren, and Chung 2017).

Usually the consumption of low amounts of biogenic amines in humans does not affect their health. In food the amount of histamine is affected by multiple factors, including the manufacturing process, the cleanliness of raw materials, microbial composition, and the duration of fermentation. Histamine is a result of the decarboxylation of the amino acid histidine. Endogenous histamine is stored primarily in mast- and basophil cells. It is an important mediator of immunoglobulin E-(IgE) and non-IgE-dependent clinical allergic reactions. The understanding of the histamine pathway in allergies and in immune regulation has led to the development of several antihistamine medications (Chazot 2013). This pathway is described with numerous pharmacogenetic variations and influences disease expression and response to treatment. Food allergies, like peanut allergy, is, with an overall low mortality, the leading cause of death related to food-induced anaphylaxis in the United States (Togias et al., 2017). HIT should be separated from food allergies and is therefore described as a non-allergic adverse reaction causing complaints of intolerance/malabsorption to ingested food (Reese et al., 2017).

Scombroid poisoning is named after the Scombridae family of fish, which includes mackerels, tunas and bonitos (Feng, Teuber, and Gershwin 2016). First descriptions of this disease were noted in association with the ingestion of these fish species (Miya et al. 2013), although it may happen with the ingestion of non-scombroid fish species as well. They naturally contain high levels of histidine, which is, with bacterial growth during e.g. improper storage, converted to histamine. Fermentation can be prevented by the fast refrigeration or freezing of fish immediately after catching (Kovacova-Hanuskova et al. 2015). Additionally, this foodborne disease related to spoiled fish also happens when cooking, smoking, or freezing does not eliminate the histamine. Scombroid toxin is reportedly the most common toxin in poisoning outbreaks associated with imported foods in the United States (Gould et al. 2017). However, scombroid poisoning is caused by the consumption of food, mostly sea animals, contaminated with bacteria that cause very high concentrations of histamine. The European Union set regulatory limits of histamine content of up to a maximum of 200 mg/kg in fresh fish and 400 mg/kg in seafood products (Visciano et al. 2014). Histamine quantity of more than 40 mg per meal (0.75 mg/kg body weight) can significantly increase the risk of poisoning (Doeun, Davaatseren, and Chung 2017).

Histamine intolerance (HIT)

The term "histamine intolerance" is used in a similar way to the term lactose intolerance, which is due to a deficiency in the enzyme lactase, as it is assumed that histamine intolerance is due to a deficiency in the enzyme diamine oxidase (DAO). HIT appears as a metabolic disease because histamine is widely distributed throughout the body (Schwelberger 2010) and it is involved in the etiology of common diseases (Kucher and Cherevko 2018).

Human DAO and histamine N-methyl transferase are enzymes which catalyze the oxidative deamination of mono-, di- and polyamines. Within the GI tract, DAO appears to be the primary enzyme for the degradation of ingested histamine (Smolinska et al. 2014). DAO is synthesized by mature intestinal enterocytes and is constantly released from the intestinal mucosa during digestion as well as into the blood circulation (Ji et al. 2013; Wollin, Wang, and Tso 1998). Impairment of the GI mucosa caused by various diseases and medications may also reduce DAO activity. DAO from the white pea (*Lathyrus sativus*) reportedly prevented histamine toxicity in vitro in human heterogeneous epithelial colorectal adenocarcinoma cells (Jumarie et al. 2017).

A disproportionate amount of histamine in the body is suspected to result from the consumption of histamine-containing food or drinks, and the reduced ability of enzymes to digest histamine. Ingested histamine, clearly below the dose of a scombroid poisoning, is thought to cause HIT-related symptoms in affected people.

The clinical diagnosis of HIT is challenging, as standardized diagnostic tests are still lacking. A thorough anamnesis of all complaints which may be linked to HIT experienced

by each individual is the mainstay of HIT diagnosis. However, diagnosis may also be supported with measurement of a serum DAO value <10 U/mL. Two or more typical GI symptoms of HIT and a reduction of complaints subsequent to a histamine-reduced diet may help to support the diagnosis. Serum DAO values are not established to correlate with gastrointestinal DAO activity. Recently, however, a significant increase of serum DAO values due to strict diet compliance was demonstrated in patients with HIT (Lackner et al. 2019). Then, evidence of correlation between low DAO values, symptoms of histamine intolerance, and response to histamine reduced diet and/or to oral diamine oxidase supplementation has been reported (Manzotti et al. 2016; Schnedl, Schenk, et al. 2019).

Currently, DAO is determined using a radio extraction assay based on the spontaneous conversion of oxidized radioactive putrescine into delta-1-pyrolline. This method seems to have limitations because only a relative amount of DAO in serum is quantified. A human DAO standard is not available and absolute DAO quantities cannot be determined. Therefore, it is necessary to develop new assay methods. An enzyme-linked immunosorbent assay (ELISA) using human DAO as a standard was found to be accurate (Boehm et al. 2017). With the widespread availability of this ELISA, the potential of DAO as biomarker for diseases, including reduced serum DAO values in HIT, needs to be reevaluated.

The search for HIT diagnostic tests continues. Double-blind placebo-controlled oral histamine provocation tests also triggered reactions in 50% of healthy controls. However, due to the lack of sufficient data, an oral histamine provocation is not suitable to diagnose HIT at present (Reese et al., 2017). Precise determination of plasma histamine values is an ongoing issue. Radioimmunoassay (RIA) is currently the gold standard method to quantify histamine. However, its limitations include the time expenditure, the specificity of the procedure for technicians and the production of radioactive waste (Poli et al. 2016; Liu et al. 2014). Another approach is the measurement of histamine and methylhistamine in urine. The widespread use of this diagnostic procedure seems limited because of the unpredictable amount of individual protein ingestion before the test (Comas-Basté et al. 2017). Histamine is also a metabolite of intestinal bacteria and this reduces the reliability of diagnostic stool analysis. Skin-prick tests have been validated for food allergy diagnoses (Gomes-Belo et al. 2018), but not for HIT (Kofler, Ulmer, and Kofler 2011).

There are known genetic polymorphisms for lactase (Järvelä, Torniainen, and Kolho 2009), celiac disease (Hunt and van Heel 2009), and a genetic background might also be a factor in fructose malabsorption (Patel et al. 2015). Additionally, polymorphisms in the genes coding for DAO (Maintz et al. 2011; Petersen, Raithel, and Schwelberger 2005) and the histamine receptors (Sadek and Stark 2016) have been identified. The polymorphisms of the four known histamine receptors and polymorphisms of DAO seem associated with multiple clinical symptoms and hundreds of symptom combinations in HIT. This may help to explain the extensive individual variability of functional, nonspecific, non-allergic GI and extra-intestinal symptoms. Furthermore, this genetic variety might influence symptoms and disease expression and individual response to diets or treatment. Although some of these polymorphisms have been identified, their functional and clinical significance remains unknown. Another factor to consider is that the histamine content in food is frequently not known (Gagic et al. 2019) and varies considerably depending on ripeness, storage time, and processing (San Mauro Martin, Brachero, and Vilar 2016). All of these variables need to be advised and may help to explain each person's unique and even sometimes changing tolerance levels concerning food intolerance/malabsorption.

Table 1. Currently available tests to be performed in each individual patient with functional, nonspecific, non-allergic abdominal complaints.

Intolerance/malabsorption	Available tests in addition to the thorough anamnesis
Lactose intolerance	Hydrogen breath test
Fructose malabsorption	Hydrogen breath test
Histamine intolerance	Serum diamine oxidase with radio extraction assay or ELISA
Celiac disease	Antibodies against tissue transglutaminase with IgA ELISA
Helicobacter pylori infection	Histologic evaluation of gastric mucosa, C13 breath test, stool antigen tests, determination of antibodies against *H.p.* with IgA ELISA

IgA ELISA, Immunoglobulin A enzyme-linked immunosorbent assay; *H.p.*, *Helicobacter pylori*

Discussion

Food intolerance/malabsorption syndromes cause non-allergic, functional, nonspecific GI complaints and scientific advances have led to a better understanding of the role of food ingredients. These functional, nonspecific, non-allergic abdominal complaints may be accompanied by extra-intestinal symptoms when a food or a combination of certain ingredients cannot be absorbed and digested properly. GI bacteria use catabolic enzymes to degrade and ferment carbohydrates and proteins from ingested food (Pimentel, Mathur, and Chang 2013; Reese et al. 2018).

Although there is limited availability of sufficiently sensitive and specific tests it seems essential to assess functional, nonspecific, non-allergic GI complaints individually with all currently available tests (Rezaie et al. 2017). All etiological factors of functional, nonspecific, non-allergic GI complaints including fructose, gluten, histamine, lactose, and *H.p.* infection need to be evaluated in each patient (Table 1). Studies have shown combined lactose and fructose intolerance/malabsorption and variable combinations of lactose-, fructose- and histamine intolerance/malabsorption, including *H.p.* infection. In an evaluation of more than 400 patients with functional, nonspecific, non-allergic GI complaints nearly 80% of the patients suffered from malabsorption/intolerance due to a single food or 7 combinations, and/or *H.p.* infection (Enko et al. 2016). After detailed diagnosis, functional, nonspecific, non-allergic GI complaints can be treated by reducing the ingestion of the triggering single or combined

nutrients and/or eradication of *H.p.* To ensure nutritional adequacy this should only be done after a detailed diagnosis and it should include the careful evaluation of the individual tolerance level to the symptomatology.

Conclusion

In conclusion, histamine seems to play a significant role in functional, nonspecific, non-allergic GI complaints. Diagnostic tools for HIT need improvement but future wide-spread availability of ELISA for determination of DAO in serum seems promising. A detailed diagnosis of single or combined food intolerance/malabsorption, including HIT, with currently available methods is necessary to evaluate patients with functional, nonspecific, non-allergic GI complaints. However, histamine-reduced diets need further evaluation, and a registered, experienced dietician is required to design an individually tailored diet. Each patient's tolerance level should be considered when recommending dietary restrictions for long-term symptom reduction. Dietary advice ought to include nutritional variety, ensure alimentary adequacy and cause negligible impact on the GI microbiome.

Disclosure statement

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Abbreviations

CD	celiac disease
DAO	diamine oxidase
EGS	eosinophilic gastroenteritis
ELISA	enzyme-linked immunosorbent assay
FD	functional dyspepsia
GI	gastrointestinal
H.p.	helicobacter pylori
HIT	histamine intolerance
IBS	irritable bowel syndrome
MCAS	mast cell activation syndrome
NCGS	non-celiac gluten sensitivity
SIBO	small intestinal bacterial overgrowth

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Research Article

Diagnostic and Clinical Significance of Serum Levels of D-Lactate and Diamine Oxidase in Patients with Crohn's Disease

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Background. Crohn's disease (CD) is a chronic intestinal inflammatory disease. An ideal laboratory marker that can predict the prognosis in terms of relapse of the disease is clinically desirable. *Methods*. A total of 59 CD patients were enrolled in this study. Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantitatively detect the content of D-lactate (D-LA) and the diamine oxidase (DAO) levels in sera obtained from patients and 28 healthy controls. The correlation between these two biomarkers and disease activity scores was assessed. In addition, the ROC curve was used to evaluate the diagnostic accuracy of these two biomarkers. *Results*. The levels of D-LA in the serum of CD patients in the active stage and remission stage were 16.08 $\pm$ 4.8 mg/L and 11.16 $\pm$ 3.17 mg/L, respectively, and the difference was statistically significant ($t = 4.67$, $P < 0.001$). DAO levels were significantly higher in patients with the active stage compared to controls. The levels of D-LA and DAO in CD patients were positively correlated with the disease activity ($r = 0.68$ and 0.53, respectively, $P < 0.05$). The area under the ROC curve (AUC) when CD activity was diagnosed with D-LA and DAO alone was 0.815 and 0.748, respectively. The diagnostic efficacy of the two biomarkers was not significantly different from that of the erythrocyte sedimentation (ESR) and hypersensitive C-reactive protein (CRP) ($P > 0.05$). However, the area under the curve was 0.861 (0.746, 0.937) when the diagnosis was performed using a combination of D-LA, DAO, CRP, and ESR, which was significantly higher than when CRP or ESR were tested alone ($P < 0.05$). *Conclusions*. D-LA and DAO have a good prognostic value for CD activity. Rational combined use of biomarkers can significantly improve the diagnostic efficiency.

1. Introduction

Crohn's disease (CD) is a chronic intestinal inflammatory disease whose etiology remains unknown. Currently, there is no cure for Crohn's disease. The goal of medical treatment is to control and maintain the intestinal inflammation within the range of a remission stage or prevent deterioration of the healing state of intestinal mucosa. The endoscopy examination with histology is mostly used to make a diagnosis, to assess the severity of disease, and to evaluate the recovery of intestinal mucosa after treatment. However, endoscopic procedure is costly, invasive, and time-consuming and it is associated with the risk of multiple complications.

An ideal laboratory marker that can detect disease activity and monitor the effectiveness of treatment, and also provide a prognostic value for relapse of the disease, is clinically desirable. Two biomarkers have been extensively studied in CD: C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). It has been shown that these two markers correlate significantly with endoscopic lesions and risk of relapse and with the response to therapy [1]. However, the sensitivity and specificity of these two markers are far from being satisfactory [2]. When CD patients suffer from a systemic infection or autoimmune connective tissue diseases, the two markers are not sufficiently accurate to detect the activity of CD, which leads to late treatment due to the inability to make a timely diagnosis.

Development of a set of new monitoring biomarkers that are more sensitive and specific is clinically important. Increasing evidence indicates that damage to the intestinal barrier increases epithelial permeability which

triggers inflammatory processes and is closely related to CD activity [3]. D-lactate (D-LA) is a product released by many of microflora residing in the human gastrointestinal tract. Thus, a rise in blood D-LA level reflects an increase in the permeability of intestinal mucosa [4]. In other words, the levels of circulating D-LA may reveal the degree of damage to the intestinal mucosa. Diamine oxidase (DAO) is synthesized only in the epithelial cells of intestinal villi. Damaged mucosal cells release DAO which increases its serum concentration. Given that activity of DAO is stable in the blood [5], the concentration of DAO in the blood may reflect the damage and restoration of the intestinal cavity in a timely manner. High concentration of D-LA and DAO in the blood is tightly linked to abnormal intestinal barrier function [6], implying that they can be used as sensitive and accurate markers for monitoring CD activity. A study has already observed that serum levels of D-LA and DAO in CD patients are markedly higher than those in normal control group and they decrease after treatment [7].

The present study was designed to assess the relationship between the concentration of D-LA, DAO, and Crohn's disease activity. It is also aimed at determining whether circulating D-LA and DAO can be used as promising molecular indicators of the activity of Crohn's disease.

2. Methods

2.1. Subjects. A total of 59 CD patients who were admitted at the Zhongda Hospital affiliated to Southeast University from October 2017 to April 2018 were included in the study. The CD diagnostic criteria were based on the *Consensus Opinions on the Diagnostic and Treatment Specifications for Inflammatory Bowel Diseases in China*. The patients were categorized by disease location (L1 ileum, L2 colon, and L3 ileocolon) according to the Montreal classification [8]. The evaluation of disease activity was carried out according to Crohn's Disease Activity Index [9]. In addition, 28 healthy individuals from the physical examination center were taken as the control group. The study was approved by the Research Ethics Committee of the Zhongda Hospital affiliated to Southeast University (Nanjing, China).

2.2. Research Method. This is a cross-sectional study. A total of 59 CD patients and 28 healthy people participated in this study. After clinical evaluations, their blood samples were obtained. The concentration of D-LA and DAO in the serum of the subjects was determined using ELISA. The ELISA kit was purchased from Beijing Zhongsheng Jinyu Diagnostic Technology Company. The concentration of ESR and CRP in the blood of patients was determined using routine testing methods. Moreover, other clinical data including loose stool times, abdominal pain degree, general situation, extraintestinal manifestations, drug use, abdominal mass, and hematocrit were weighted and collected, and the CDAI score of each patient was calculated. The remission stage was defined as CDAI < 150 points, and the active disease stage was defined as CDAI ≥ 150 points.

2.3. Statistical Analysis. Statistical analysis was performed using SPSS 23.0 and MedCalc 15.8 software. The measurement data conforming to the normal distribution is presented as $x \pm s$, and the t-test was used for comparison between two groups of independent sample. Analysis of variance was used to compare multiple groups of data. The Pearson correlation coefficient was used to assess the correlation between the two variables. The ROC curve was used to evaluate the diagnostic efficacy, and the areas under the ROC curve were compared using Z-test. The group differences were considered statistically significant at the 5% level ($P < 0.05$).

3. Results

3.1. Characteristics of Study Subjects. A total of 59 patients were included in the study. Among them, 29 were in the active stage and 30 were in the remission stage. Patients who were in the active stage aged between 20 and 47 years old, with an average age of 37.2 ± 8.3 years old (including 13 males and 16 females). Patients in the remission stage aged between 17 and 55 years old, with an average age of 39.6 ± 13.3 years old (including 17 males and 13 females). The average age of the control group was 43.6 ± 13.8 years old (including 13 males and 15 females). There was no significant difference in sex, age, and other basic characteristics among the groups ($P > 0.05$) (Table 1).

3.2. Analysis of Levels of D-LA and DAO. The serum levels of D-LA in CD patients who were in the active and remission stages were 16.08 ± 4.8 mg/L and 11.16 ± 3.17 mg/L, respectively, and the difference between the two groups was statistically significant ($t = 4.67$, $P < 0.001$) (Table 2). There was no significant difference in serum D-LA levels between the remission stage CD patients and the healthy controls (10.2 ± 3.22 mg/L) ($t = 1.13$, $P > 0.05$). The serum levels of DAO in CD patients who were in the active and remission stages were 11.01 ± 4.49 U/L and 7.7 ± 3.44 U/L, respectively, and the difference between the two groups was statistically significant ($t = 3.18$, $P < 0.05$) (Table 2). However, there was no significant difference in serum DAO levels between the remission stage CD patients and the healthy controls (6.21 ± 2.34 U/L) ($t = 1.91$, $P > 0.05$).

3.3. Correlation between D-LA, DAO, and CD Disease Activity. According to the Pearson correlation analysis, the serum level of D-LA in CD patients was correlated with disease activity scores ($r = 0.68$, $P < 0.05$). The serum level of DAO in CD patients was also correlated with the disease activity scores ($r = 0.53$, $P < 0.05$). Moreover, CRP and ESR were correlated with the disease activity scores ($r = 0.58$ and 0.46, respectively, $P < 0.05$) (Figure 1).

3.4. Accuracy and ROC Analyses of D-LA, DAO, ESR, and CRP. According to the ROC curve, it was observed that serum D-LA and DAO have prognostic values for CD activity ($P < 0.05$). This was the case for the traditional biomarkers:

Table 1: General characteristics of 59 patients with CD.

Characteristics	CD active ($n = 29$)	CD remission ($n = 30$)	P value
Age in years, mean (range)	37.2 (20-47)	39.6 (17-55)	0.408
Sex: male, n (%)	13 (45)	17 (57)	0.363
Duration of disease in years, median (IQR)	7 (2-11)	7.5 (3-13)	0.504
Smoking history, n (%)			0.626
Current	8 (27)	6 (20)	
Former	10 (34)	9 (30)	
Never	11 (38)	15 (50)	
Site of disease, n (%)			0.605
Ileal (L1)	2 (7)	1(3)	
Colonic (L2)	13 (45)	17(57)	
Ileocolonic (L3)	14 (48)	12(40)	
Drugs before inclusion, n (%)			0.450
Mesalamine	5 (17)	3 (10)	
Azathioprine/6-MP	7 (24)	5 (17)	
Anti-TNF	10 (34)	9 (30)	
Anti-TNF and azathioprine/6-MP	7 (24)	13 (43)	
Number of prior surgeries, mean (range)	0.20 (0-2)	0.28 (0-3)	0.898
Having extraintestinal manifestations, n (%)	2 (6)	1 (3)	0.612

CD: Crohn's disease; 6MP: 6-mercaptopurine; IQR: interquartile range. There were no significant differences between groups in the active stage CD patients and remission stage CD patients.

ESR and CRP. The Youden index of each biomarker when they were used to diagnose CD activity was calculated. The maximum value reflected the evaluation accuracy. At 14.91 mg/L, the sensitivity and specificity of D-LA in diagnosing disease activity were 69% and 93%, respectively. At 9.75 U/L, the sensitivity and specificity of DAO in diagnosing disease activity were 76% and 77%, respectively. The area under ROC curve of the four biomarkers of CD activity was D-LA, CRP, DAO, and ESR in a descending order. All areas under ROC curve were compared with Z-test. There were no statistically significant differences between the groups ($P > 0.05$). Also, we applied the logistic regression model to generate combined predictors (Comb) as the analysis index. When D-LA, DAO, CRP, and ESR were used in combination with diagnosis, the corresponding area under the ROC curve was 0.861 (0.746, 0.937), which was significantly higher than ESR and CRP when they were used alone to evaluate CD activity ($P < 0.05$). Therefore, combining predictors relatively improved the diagnostic sensitivity and specificity (Table 3 and Figure 2).

4. Discussion

CD is a chronic intestinal inflammation and its pathogenesis remains unclear. Accumulating evidence suggests that disruption of the intestinal barrier function is associated with the occurrence and development of CD [10]. The mechanical barrier is the most important part of the intestinal mucosal barrier, and it is the histological foundation that maintains intestinal barrier function. It prevents the entry of various harmful substances and pathogen into the intestine [11]. The integrity of the mechanical barrier is regulated by the tight junctions between intestinal epithelial cells. The tight junctions are mainly composed of various intracellular proteins such as occlusive protein and filamentous actin. Studies have shown that the expression of these proteins in the junctional complex of colonic mucosa in CD patients is downregulated compared with that in normal people [12], which weakens the mechanical barrier and in turn induces inflammatory bowel disease. Studies have also shown that intestinal crypt epithelial cell apoptosis is positively correlated with the degree of colonic inflammation [13]. Microbial barriers are mainly associated with the intestinal flora. Recent advances in the use of genetic detection tools have increased our understanding on the role of the intestinal flora in CD. The microbial barrier formed by the intestinal flora can suppress CD progression. It has been reported in some studies that the use of probiotics or certain bacterial precursors can improve CD [14]. Kruis et al. suggested that nonpathogenic *Escherichia coli Nissle 1917* induced remission of intestinal inflammation and displayed high curative efficacy equivalent to that of mesalazine [15]. Butyrate and short-chain fatty acids are products of anaerobic fermentation in the bowel. They reduce intestinal PH levels and provide energy for colonic epithelial cells. However, the bacterial composition of the intestines of patients with CD is altered, resulting in lower levels of butyrate and short-chain fatty acids. This impairs the metabolism of intestinal epithelial cells, which in turn damages epithelial cells and ultimately induces intestinal inflammation [16]. The studies mentioned above have shown that destruction of the microbial barrier is closely related to the occurrence of CD.

Some prospective studies have suggested that intestinal mucosal permeability can be used as an indicator of inflammatory bowel disease. Tibble et al. demonstrated, through a prospective clinical study on 43 CD patients, that the increase in intestinal mucosal permeability of patients with active CD will increase the probability of complications and recurrence [17]. In recent years, many biomarkers have been used to evaluate the intestinal barrier function in various studies. These biomarkers can be divided into three main categories. The first category contains substances found in the intestinal epithelial cells which are used to directly reflect the extent of intestinal mucosal damage. There are also some biomarkers that can reveal the damage of intestinal barrier indirectly. Such biomarkers are released into the blood when the intestinal barrier is damaged. Moreover, there are some nonspecific indicators of inflammation that are connected to intestinal mucosal damage. Among the biomarkers listed above, DAO and D-LA have potential clinical application value in the diagnosis of CD activity.

DAO, a key enzyme that catalyzes the oxidation of histamine diamine, is mainly produced in the human small intestine mucosa. The activity of DAO can reflect the functional condition of small intestines [18]. The epithelial cells at the site of damage will release a large amount of DAO. DAO is

Table 2: Comparison of levels of D-LA and DAO among groups.

Group	CD active ($n = 29$)	CD remission ($n = 30$)	P value*	Control group ($n = 28$)	P value*
D-LA (mg/L)	16.08 ± 4.8	11.16 ± 3.17	<0.001	10.2 ± 3.22	<0.001
DAO (U/L)	11.01 ± 4.49	7.7 ± 3.44	<0.05	6.21 ± 2.34	<0.001

CD: Crohn's disease; D-LA: D-lactate; DAO: diamine oxidase; *Compared with CD active.

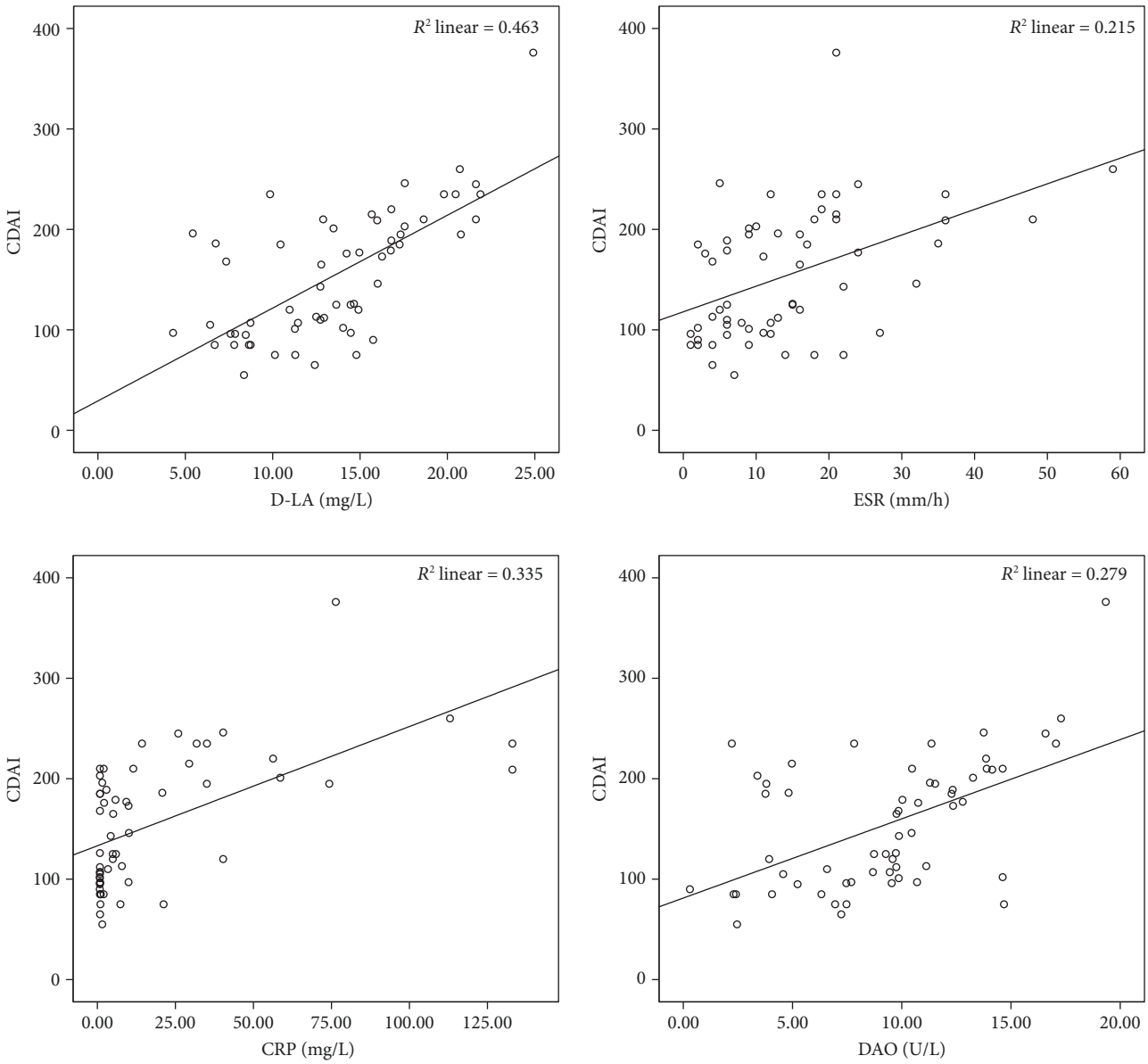

Figure 1: Correlation analysis of D-LA, DAO, ESR, CRP, and CDAI score.

stably in serum, and therefore, it can reflect the situation of intestinal barrier [19]. D-LA is derived from the metabolism of natural flora within the gastrointestinal tract, and a variety of intraintestinal bacteria can produce D-LA through the glycolysis pathway. The permeability of the intestinal barrier increases when the intestinal barrier is damaged. As a result, D-LA produced by bacteria is released into the blood. Therefore, the serum level of D-LA increases, thereby indicating abnormal intestinal permeability [20]. Mammals do not express D-LA dehydrogenase. Thus, it is reliable to select this biomarker to evaluate intestinal barrier function.

The results of this study showed that the levels of D-LA and DAO in active stage CD patients were higher than those in the remission stage, suggesting that these serological biomarkers can be used to determine whether CD is active or not. This study also found that the serum levels of D-LA and DAO in CD patients in the remission stage were not significantly different from those in healthy population,

Table 3: Accuracy and ROC analyses of D-LA, DAO, ESR, and CRP in differentiating CD active and CD remission.

Diagnostic index	AUC (95% CI)	Standard error	*P* value	Youden (max)	Cut value	Sensitivity	Specificity
D-LA	81.5 (0.692, 0.904)*	0.06	<0.001	0.623	14.91 mg/L	68.97	93.33
DAO	74.8 (0.618, 0.852)△	0.07	<0.001	0.525	9.75 U/L	75.86	76.67
ESR	70.5 (0.572, 0.816)	0.07	<0.05	0.386	15 mm/h	58.62	80.00
CRP	78.4 (0.658, 0.881)	0.06	<0.001	0.487	7.85 mg/L	62.07	86.67
Comb	86.1 (0.746, 0.937)#	0.05	<0.001	0.659	0.47	75.86	90.00

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; D-LA: D-lactate; DAO: diamine oxidase; AUC: area under the curve; Comb: combined predictors. Compared with ESR and CRP, #$P < 0.05$; compared with ESR and CRP, *$P > 0.05$; compared with ESR and CRP, △$P > 0.05$.

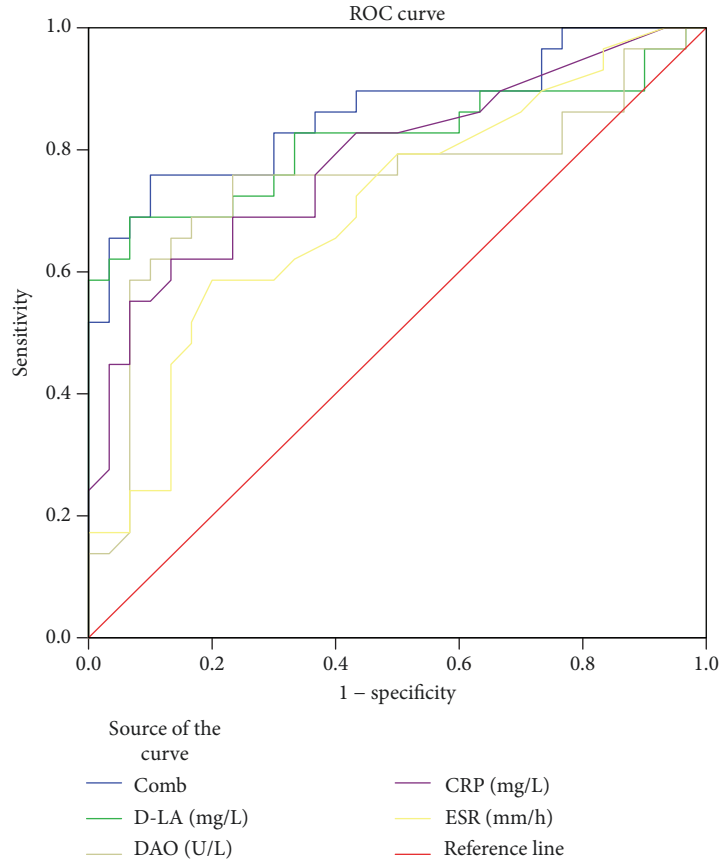

Figure 2: Receiver operating characteristic curves of the various biomarkers in CD active compared to CD remission.

suggesting that these biomarkers are not appropriate in the diagnosis of CD, but they are only valuable in identifying the active and remission stages of CD. The most common method used to evaluate CD disease activity is the CDAI scoring system. This study found that D-LA and DAO levels in CD patients were strongly correlated with CDAI scores, and the relationship between D-LA and disease activity score was stronger than CRP and ESR, which are currently used in clinical practice. This finding further suggests that detection of D-LA and DAO levels is more effective for evaluating CD activity. According to the ROC curve, D-LA and DAO displayed good diagnostic value when used alone to detect CD activity. Their diagnostic efficacy is not significantly different from that of ESR and CRP. We also calculated the Youden index, and the maximum value was used as the index of accuracy evaluation. When D-LA takes the cut value, the sensitivity and specificity for diagnosing disease activity are 69% and 93%, respectively, which are higher than ESR and CRP. In addition, detection of D-LA and DAO in combination with CRP and ESR significantly improved the diagnostic efficiency, with better sensitivity and specificity.

Accumulating evidence demonstrates that intestinal mucosa healing can reduce clinical recurrence rate, hospitalization rate, and operation rate [21]. Endoscopic score is used to evaluate CD therapy. Twelve patients in this study underwent endoscopy by the same doctor, and Simplified Endoscopic Score for Crohn's Disease (SEC-CD) scores were obtained in strict compliance with the scoring system. However, only 3 patients were in the active stage, and no conclusion with statistical difference can be obtained. Further research is required to address this question.

In clinical practice, disease biomarkers are applied in the control of diseases. Mucosal healing usually reflects the control level of acute inflammatory activity, which indirectly reflects the efficacy of drugs [22]. In this study, we did not evaluate the changes of these biomarkers before and after treatment, but we believe that D-LA and DAO, indicators of intestinal mucosal injury, have a great potential in predicting the efficacy of medical treatment of the disease. Further studies are necessary to validate this finding.

In summary, serum D-LA and DAO possess high diagnostic value for CD activity, and when they are used in combination with the currently used biomarkers, CRP and ESR, their diagnostic accuracy for CD activity was significantly improved. However, the sample size of this study is small. Additional investigations with large sample size are needed to support the results of this study. Moreover, it should be noted that these two indexes have not been compared with the endoscopic scores of CD due to the limitation of sample size. We believe that this study provides valuable information that can help to identify Crohn's disease activity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All the authors have no conflicts of interest to declare.

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Microbial patterns in patients with histamine intolerance.

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MICROBIAL PATTERNS IN PATIENTS WITH HISTAMINE INTOLERANCE

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Histamine intolerance represents a controversially discussed disorder. Besides an impaired degradation of orally supplied histamine due to diamine oxidase (DAO) deficiency, a deranged gut flora may also contribute to elevated histamine levels. Our aim was to determine the intestinal bacterial composition in patients with proven histamine intolerance in comparison to other food intolerances and healthy controls. A total of 64 participants were included in the study, encompassing 8 patients with histamine intolerance (HIT), 25 with food hypersensitivity (FH), 21 with food allergy and 10 healthy controls (HC). All participants underwent blood testing for total and food-specific immunoglobulin E, plasma histamine and DAO serum activity. Stool samples were used to analyze stool histamine and zonulin levels and bacterial composition by 16s rRNA sequencing. No significant differences in stool histamine levels were observed, but HIT patients showed elevated levels of stool zonulin. Microbiota analysis revealed increased levels of Proteobacteria (5.4%) and a significantly reduced alpha-diversity in the HIT group ($P = 0.019$). On family level, HC showed a significantly higher abundance of Bifidobacteriaceae compared to other study groups ($P = 0.005$), with lowest levels in the HIT group ($P = 0.036$). Also significantly reduced abundances of the genera Butyricimonas ($P = 0.026$) and Hespellia ($P = 0.025$) were observed in the HIT patients, whereas Roseburia were significantly elevated ($P = 0.021$). We concluded that the altered occurrence of Proteobacteria and Bifidobacteriaceae, reduced alpha-diversity as well as elevated stool zonulin levels suggest a dysbiosis and intestinal barrier dysfunction in histamine intolerant patients, which in turn may play an important role in driving disease pathogenesis.

Key words: *dysbiosis, food intolerance, gastrointestinal microbiome, histamine, intestinal barrier, diamine oxidase, lactic acid bacteria*

INTRODUCTION

The prevalence of patients suffering from gastrointestinal and extra-intestinal afflictions after food ingestion is rising. The spectrum of food intolerances reaches from carbohydrate malabsorption (*e.g.* lactose, fructose) to immunological IgE or non-IgE-mediated food allergies (1, 2). In addition, histamine intolerance (HIT) is also often considered to be responsible for gastrointestinal symptoms after food intake. Thereby, histamine intolerance is defined as an adverse reaction of ingested histamine that affects different organ systems and results in various intestinal and extra-intestinal symptoms (3). Ingestion of histamine containing foods and beverages, including fish, cheese or red wine, are supposed to trigger symptoms like flush, pruritus, nausea, vomiting, diarrhea and abdominal pain (3). Other foods like citrus fruits or various drugs further contribute to an elevated histamine concentration through their histamine-liberating effect (4). Although the exact mechanism of the pathogenesis is still unclear, a reduced intestinal diamine oxidase (DAO) activity, which is important for degradation of exogenously supplied histamine, is presumed (4). This leads to an insufficient degradation of food derived histamine, which passes into the blood stream leading to increased plasma histamine concentrations and evoking the described symptoms by affecting various organ systems (*e.g.* cardiovascular system, respiratory tract, skin, nervous system, intestine) (4, 5).

However, also other factors are discussed to affect histamine intolerance, for example an alteration of the intestinal bacteria. Various bacteria, which are able to convert histidine from proteins into histamine, naturally occur in the digestive tract as part of the normal intestinal gut flora (6, 7).

Interestingly, some probiotic strains including several lactic acid bacteria, like *Lactobacillus reuteri*, *Lactobacillus casei* and *Lactobacillus delbrueckii subsp. bulgaricus*, possess the enzyme histidine decarboxylase (HDC) and are therefore able to generate biogenic amine (8, 9). The presence of these bacteria in the human intestine might contribute to increased histamine levels and promote histamine sensitivity in some persons.

It is well known that alterations of the human intestinal microbiota are linked to various diseases. Besides obesity or cardiovascular disease (10, 11), a dysbiosis is discussed in the pathogenesis of different autoimmune diseases including type 1 diabetes, rheumatoid disease, inflammatory bowel disease or celiac disease (12, 13). But even in patients with an allergy, the influence of the microbiota as a triggering factor for asthma and food allergy is discussed (14, 15). Several studies revealed a

correlation between a low microbial exposure in childhood and an increased risk for allergies. Thereby, multiple factors can influence this risk positively or negatively by altering the intestinal microbiota, *e.g.* mode of birth, duration of breastfeeding, treatment with antibiotics, infections, living with older siblings or furred pets (16). Interestingly, the use of probiotics seems to have immunomodulatory effects in allergic disease by suppressing histamine signaling (17). *Via* the induction of regulatory T cells (Tregs) some Clostridia species seem to suppress symptoms in murine models of intestinal allergy (18). All these facts underline the importance of intestinal bacteria in human immunity and health.

To determine the influence of the human gut microbiota in the pathogenesis of histamine intolerance, we analyzed the intestinal bacterial composition by sequencing the bacterial 16S rRNA of stool samples derived from patients with a confirmed diagnosis of histamine intolerance. These data were compared with the microbial patterns of stool samples from healthy individuals, patients with food allergy or food hypersensitivity. The measurement of histamine concentrations in stool samples was done to evaluate the histamine production by intestinal bacteria. To assess the gut permeability, zonulin, a regulator of tight junctions, was measured in serum and stool samples.

MATERIALS AND METHODS

Study participants

Patients with histamine intolerance, food hypersensitivity and allergies were recruited over a 12-month period *via* the outpatient clinic for nutritional medicine of the Medical Department 1 of the University Hospital Erlangen as well as social media platforms. Healthy controls were recruited from the circle of friends or colleagues. A total of 64 patients were included in the study. Exclusion criteria were pregnancy, lactation, being underage and current intake of antibiotics, anti-histamines or anti-inflammatory medication.

All participants were informed in detail by a doctor about the aim and procedure of the study and gave their written informed consent prior study inclusion. The study was approved by the ethics committee of the Friedrich-Alexander-University Erlangen (application number: 231_14B) and in accordance with the declaration of Helsinki.

Group allocation

Blood samples were taken of all study participants to determine total immunoglobulin E (IgE) as well as ten food-specific IgEs (chicken's egg white, milk protein, wheat flour, rye flour, nut mixture, soy bean, tomatoes, salmon, casein and celeriac). Participants with gastrointestinal (diarrhea, nausea, vomiting, abdominal pain) and extra-intestinal symptoms (allergic rhinitis, oral allergy syndrome, headache, fatigue, skin changes, asthmatic symptoms) briefly after food ingestion and positive serological food-specific IgE antibodies and significantly elevated total IgE (361.2 kUA/L; $P > 0.001$) were classified as food allergy patients (FA group). Individuals with symptoms, but negative food-specific IgE antibodies and low total IgE levels (< 100.0 kUA/L) underwent further measurements of plasma histamine levels and serum DAO activity. Patients with impaired histamine degradation, characterized by elevated plasma histamine levels and decreased DAO activity in serum and an alleviation of symptoms during a histamine-free diet, were further validated by repeated blood samples over a period of 24 hours, and allocated to the histamine intolerant group (HIT group). The remaining participants without IgE antibodies and without validated histamine intolerance, but clinical symptoms including abdominal pain, diarrhea, nausea, headache, skin changes or allergic rhinitis, were classified as food hypersensitive patients (FH group). The healthy controls (HC group) showed no clinical symptoms and no signs for food allergy and histamine intolerance.

The whole diagnostic procedure for group allocation was published by Pinzer *et al.* (19).

Nutritional assessment

To determine daily intake of macronutrients the Freiburger Diet Protocol (Nutri-Science GmbH, Freiburg, Germany) was used. Therefore, all study participants were asked to fill in a three-day nutritional dairy at the beginning of the study. Daily intake of energy, carbohydrates, fats, fibers and alcohol was evaluated by PRODI® (version 6.5 expert, Nutri-Science GmbH).

Sample collection and analysis

Venous blood samples were taken from every study participant and plasma histamine, DAO activity, total IgE concentration and food-specific IgE antibodies were quantified in blood serum. Serum zonulin and TNF-α concentrations were measured by ELISA (IDK® Zonulin ELISA Kit and IDK® TNF-α ELISA, Immunodiagnostic AG, Bensheim, Germany) following manufacturer's instructions. Histamine and zonulin from stool samples were determined with the Histamine ELISA and IDK® Zonulin ELISA from Immunodiagnostic AG, Bensheim, Germany. Stool samples were collected once at study beginning from all study participants and were immediately cooled at 4ºC and stored within 4 hours at –20ºC till analysis. Bacterial DNA was isolated with the QIAamp Fast DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) following manufacturer's instructions. DNA concentration was measured by NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, USA).

For 16S-based microbiome analysis, the amplification of the V3-V4 region of bacterial 16S rRNA was realized by NEBNext Q5 Hot Start Hifi PCR Master Mix (New England Biolabs, Ipswich, USA). Amplicons were purified with AMPure XP Beads (Beckman Coulter Genomics, Indianapolis, USA), and the DNA content was measured by fluorometric quantitation using the Qubit® dsDNA-Kit (Thermo Fisher Scientific, Germany). DNA samples were pooled and analyzed by 2 × 300 bp paired-end sequencing on the Illumina MiSeq platform. Quality control, OTU table generation and taxonomic classification against the database of the 'Ribosomal database projects' (RDP, version 16) was performed using Usearch 10 (64 bit version).

Statistical analysis

Statistical analyses were performed using SPSS version 21 (IBM SPSS Statistics, Ehningen, Germany) and GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). Bioinformatics analysis for bacterial composition were performed with METAGENassist (20) and MicrobiomeAnalyst (21). Characteristic data are described as means ± standard deviation (SD), median and range (min-max) or in number (n) and percent (%). All data for bacterial proportions are described as median with minimum to maximum values (min-max). For statistical evaluation data were checked by Kolmogorov-Smirnov-test for normal distribution. Differences between study groups were determined using Kruskal-Wallis-test for non-parametric data. Due to the exploratory character of this pilot study, no correction

for multiple testing was applied. For categorical variables differences between study groups were analyzed by Pearson's chi-squared test. The alpha-diversity was described using Shannon-Weaver-Index (SWI) and Simpson's reciprocal index (SI). The Permutational Multivariate Analysis of Variance (PERMANOVA) was used for analysis of pairwise inter-sample distances with Bray-Curtis method. Correlation analysis of variables was computed using the non-parametric Spearman rank correlation.

All tests for significance were two sided, and a P-value of $P < 0.05$ was considered as statistically significant.

RESULTS

Characteristics

Overall 64 study participants (38.3 ± 14.2 years, 84.4% female) were enclosed to the study, and classified according to a previous study (19). Briefly, 33 patients (age 38.4 ± 13.4 years, 84.8% female) had suspected histamine intolerance. The 24 h histamine profiling and the measurement of serum DAO activity revealed 8 out of these 33 patients (12.5%) with histamine intolerance by definition (decreased DAO activity) and these patients were allocated to HIT-group. The remaining 25 patients (age 41.4 ± 12.8 years; 80.0% female) with normal DAO activity were considered as food hypersensitive (FH-group). Additionally, 21 patients with proven food allergy (age 41.4 ± 14.9 years, 81% female) and 10 healthy volunteers (age 31.3 ± 13.9 years, 90% female) without gastrointestinal complaints were acquired.

Patients with proven food allergy showed positive IgE antibodies against nut mixture (76.2%), wheat flour (47.6%), celery (42.9%), tomato (23.8%), rye flour (23.8%), soybean (14.3%), and milk protein (4.8%) as well as significantly increased total IgE levels (361.2 ± 911.2 kUA/l; $P < 0.001$). None of the individuals from the other groups showed specific IgE antibodies against foods.

The presence of further comorbidities including asthma, atopic eczema, cardiovascular disease, depression, endometriosis, fibromyalgia, gastrointestinal disease, hypothyreodism was not significantly different between all study groups.

Patients characteristic are shown in *Table 1*.

Serum and stool parameters

Patients in the HIT group revealed elevated levels of TNF-α compared to other study groups ($P = 0.097$) (*Table 1*). However, only one HIT patient showed a serum TNF-α concentration above the reference threshold value of 20 ng/ml (*Fig. 1a*). Zonulin levels in stool and serum were similar in all participants ($P = 0.726$ and $P = 0.595$) (*Fig. 1b* and *1c*), with highest median levels for stool zonulin in patients that belong to the HIT or FH groups (*Table 1*, *Fig. 1b*). Concerning the stool histamine concentrations one patient of the FH and one patient of the FA group showed very high stool histamine levels (> 24.000 ng/ml) (*Fig. 1d*). The

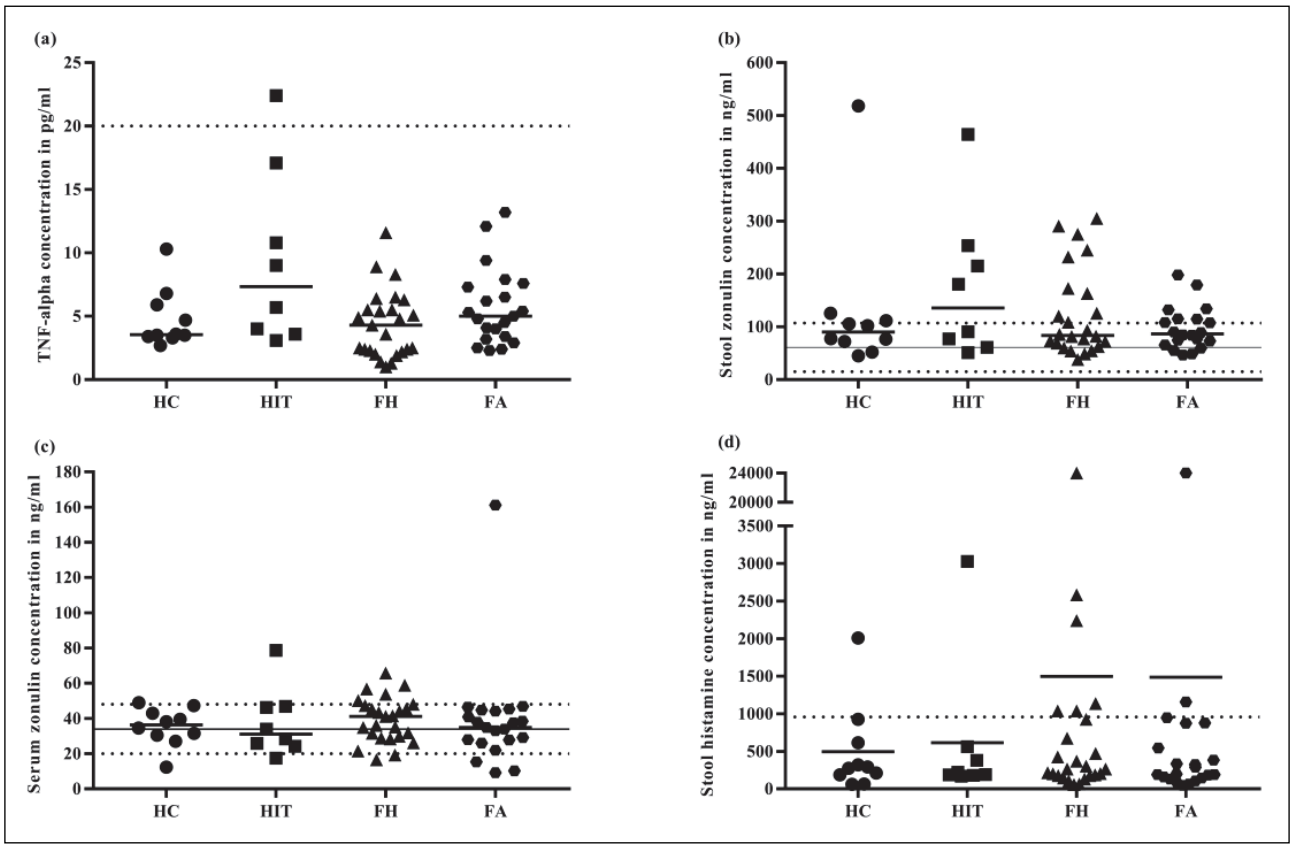

Fig. 1. Blood and stool parameters of study groups. *Fig. 1a* shows individual serum TNF-α concentrations with mean values. The dotted line indicates the reference threshold for normal TNF-α values (< 20 pg/ml). *Fig. 1b* and *1c* show individual zonulin concentrations in stool (*b*) and serum (*c*) with median (horizontal line). The solid lines mark the reference medians and dotted lines indicate the under and upper threshold of normal concentrations. *Fig. 1d* shows individual histamine stool concentrations of study groups with mean values (horizontal line). Dotted line indicates reference threshold for normal histamine values (< 959 ng/ml). Kruskal-Wallis test was used for multiple comparisons of laboratory values between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

Table 1. Patient characteristics of study groups.

Characteristic	HC	HIT	FH	FA	P-value
Demographic					
Amount [n(%)]	10 (15.6)	8 (12.5)	25 (39.1)	21 (32.8)	–
Age [years]	31.3 ± 13.9	28.9 ± 11.2	41.4 ± 12.8	41.4 ± 14.9	**0.026***
Male [n(%)]	1 (10.0)	0 (0.0)	5 (20.0)	4 (19.0)	0.518
Female [n(%)]	9 (90.0)	8 (100.0)	20 (80.0)	17 (81.0)	
Body mass index [kg/m²]	21.2 ± 2.0	24.6 ± 6.5	24.2 ± 4.6	23.4 ± 4.1	0.273
Alcohol consumption [n(%)]	8 (80.0)	6 (75.0)	15 (60.0)	17 (81.0)	0.397
Nicotine abuse [n(%)]	3 (30.0)	1 (12.5)	2 (8.0)	3 (14.3)	0.411
Probiotic use [n(%)]	0 (0.0)	0 (0.0)	4 (16.0)	1 (4.8)	0.248
Comorbidities					
Asthma [n(%)]	0 (0.0)	0 (0.0)	3 (12.0)	3 (14.3)	0.447
Atopic eczema [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	3 (14.3)	0.432
Cardiovascular disease [n(%)]	1 (10.0)	0 (0.0)	2 (8.0)	1 (4.8)	0.805
Depression [n(%)]	0 (0.0)	1 (12.5)	2 (8.0)	0 (0.0)	0.357
Endometriosis [n(%)]	0 (0.0)	1 (12.5)	0 (0.0)	2 (9.5)	0.271
Fibromyalgia [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)	0.359
Gastrointestinal disease [n(%)]	0 (0.0)	1 (12.5)	3 (12.0)	1 (4.8)	0.578
Hypothyreodism [n(%)]	0 (0.0)	2 (25.0)	4 (16.0)	6 (28.6)	0.265
Migraine [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)	0.359
Serum and stool parameters					
Total IgE [kUA/l]	14.3 ± 9.6	38.7 ± 29.9	25.9 ± 22.7	361.2 ± 911.2	**> 0.00**
TNF-α [pg/ml]	3.6 (2.7 – 10.3)	7.4 (3.1 – 22.4)	3.6 (1.0 – 11.6)	5.0 (2.3 – 13.2)	0.097
Zonulin serum [ng/ml]	36.4 (12.3 – 48.9)	31.2 (17.5 – -78.7)	41.2 (16.6 – 65.8)	34.9 (9.2 – 161.2)	0.595
Zonulin stool [ng/ml]	90.0 (45.1 – 518.2)	135.7 (51.2 – 464.2)	85.7 (38.0 – 305.0)	84.6 (46.8-198.3)	0.726
Histamine stool [ng/ml]	283.7 (62.6 – 2008.0)	206.0 (169.8 – 3027.5)	265.2 (57.9 – 24000.0)	196.6 (45.7 – 24000.0)	0.828
Nutritional intake					
Energy intake [kcal/d]	2081.7 ± 818.6	2431.3 ± 362.3	2873.3 ± 1218.1	2862.9 ± 1552.8	**0.025***
Carbohydrates [g/d (%TE)]	232.2 ± 104.2 (44.5)	302.4 ± 69.4 (50.4)	291.4 ± 112.1 (40.9)	258.3 ± 94.0 (42.1)	0.097
Fat [g/d (%TE)]	85.9 ± 34.6 (37.6)	86.1 ± 15.9 (31.8)	126.9 ± 66.3 (38.4)	103.5 ± 34.1 (37.1)	0.059
Protein [g/d (%TE)]	79.6 ± 33.0 (15.3)	87.1 ± 24.9 (14.5)	116.1 ± 51.4 (16.6)	94.1 ± 28.2 (16.1)	**0.026***
Fiber [g/d (%TE)]	19.4 ± 2.5 (1.9)	31.7 ± 0.0 (1.9)	68.3 ± 81.5 (2.2)	22.8 ± 12.6 (1.7)	0.261
Alcohol [g/d (%TE)]	4.3 ± 2.4 (0.7)	5.0 ± 6.2 (1.0)	13.6 ± 14.7 (2.0)	15.3 ± 9.9 (3.2)	**0.028***

Data are presented as number and proportions (%), mean ± standard deviation. Laboratory values (except total IgE) are expressed as median and range (min to max). Comparisons between HC, HIT, FH and FA group are assessed by Pearson's chi-squared test, respectively, for categorical variables and Kruskal-Wallis test for continuous variables. Statistically significant differences are indicated by *P < 0.05; ***P < 0.001 and marked in bold type. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers; %TE, Total energy percent.

analysis of stool histamine concentrations revealed no significant differences between study groups (P = 0.828) (*Table 1*).

Nutritional intake

The nutritional assessment revealed significant differences in the daily protein (P = 0.026) and alcohol (P = 0.028) intake between the study groups (*Table 1*). Our healthy controls ingested lower amounts of protein, reaching significance compared to the FH group (P = 0.005). Both, patients of the HIT group and healthy controls, consumed significantly less alcohol than patients of the FA group (P = 0.013 and P = 0.023). Daily carbohydrate intake was highest in the HIT group.

Correlation analysis

Correlation analysis between measured blood and stool parameters *via* Spearman rank method revealed significant correlations. Thereby the stool concentrations of histamine and zonulin showed a moderate positive correlation (r = 0.454; P < 0.001). Both, parameters for α-diversity, SWI (r = 0.339; P = 0.007) and SI (r = 0.337; P = 0.007), were correlated with TNF-α concentrations.

Microbiome analysis

To compare the microbial composition between different study groups, we converted the bacterial counts into percentages. Bacterial phyla, families and genera with an overall percentage below 0.01% were excluded from analysis.

The microbial patterns showed differences between all study groups. On phylum level, Bacteroides (61.9%), Firmicutes (31.7%) and Proteobacteria (3.7%) were most abundant in all study participants. Significant differences were observed for Verrucomicrobia (P = 0.030) with elevated numbers in patients with FH [0.35% (0.0 – 16.4%)], but minor proportions in HC [0.02% (0.00 – 2.36%)], HIT [0.00% (0.00 – 0.07%)] and FA groups [0.08% (0.00 – 3.05%)], respectively (*Fig. 2a*, *Table 2*). Interestingly, patients from the HIT group showed very low levels of Verrucomicrobia, without any abundance in five patients reaching significance to FH and FA group (P = 0.003 and P = 0.019). In contrast, HIT patients had elevated proportions of Proteobacteria [5.36% (1.34 – 34.59%)] compared to the other study groups, although significance was not reached, because of great variations between the HIT patients (*Fig. 2b*, *Table 2*).

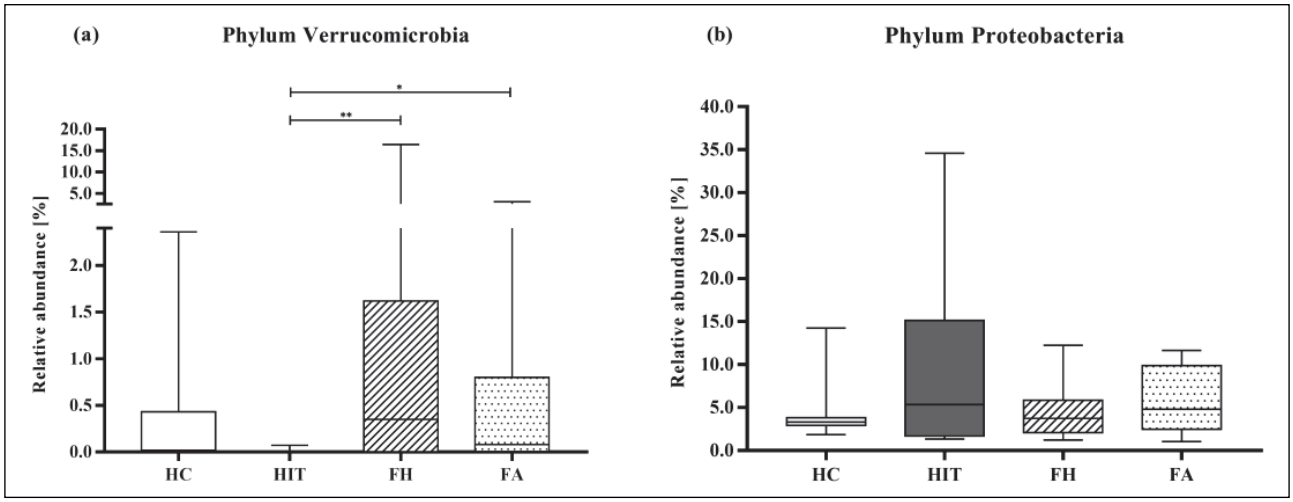

Fig. 2. Differences on phylum level between study groups. *Fig. 2a* shows significant higher relative abundance of the phylum Verrucomicrobia in FH and FA group compared to the HIT group (P = 0.003 and 0.019). *Fig. 2b* showed highest abundance for the phylum Proteobacteria in the HIT group [5.36% (1.34 – 34.59%)]. Kruskal-Wallis test was used for multiple comparisons between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers. Significance: *P < 0.05 and **P < 0.01.

Table 2. Phylum level - relative abundance.

Phylum	HC	HIT	FH	FA
Bacteroidetes	56.94 (37.94 – 74.03)	63.97 (15.80 – 86.00)	63.94 (27.56 – 83.22)	61.19 (27.56-83.22)
Firmicutes	37.00 (22.29 – 54.78)	25.51 (12.43 – 46.50)	28.87 (14.40 – 58.82)	34.07 (10.63-53.84)
Proteobacteria	3.33 (1.85 – 14.25)	5.36 (1.34 – 34.59)	3.75 (1.22 – 12.26)	4.82 (1.04-11.64)
Actinobacteria	0.45 (0.06 – 3.31)	0.12 (0.01 – 7.39)	0.25 (0.03 – 3.97)	0.13 (0.07-2.86)
Verrucomicrobia	0.02 (0.00 – 2.36)	**0.00 (0.00 – 0.07)†,††**	**0.35 (0.00 – 16.44)††**	**0.08 (0.00-3.05)†**
Tenericutes	0.0013 (0.00 – 0.92)	0.00 (0.00 – 0.00)	0.00 (0.00 – 2.32)	0.00 (0.00-4.45)
Synergistetes	0.00 (0.00 – 0.28)	0.00 (0.00 – 0.13)	0.00 (0.00 – 0.72)	0.00 (0.00-0.03)
Lentisphaerae	0.00 (0.00 – 0.20)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.93)	0.00 (0.00-0.35
Acidobacteria	0.00 (0.00 – 0.01)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.19)	0.00 (0.00-2.26)
Elusimicrobia	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 2.04)	0.00 (0.00-0.00)

Data are presented as median and range (min-max). Kruskal-Wallis test was used for multiple comparisons between study groups. Statistically significant differences are indicated by P < 0.05 and marked in bold type. Significance: †P<0.05, ††P<0.01 comparison between HIT, FH or FA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

Table 3. Family level - relative abundance.

Family	HC [%]	HIT [%]	FH [%]	FA [%]
Bacteroidaceae	32.24 (10.61–58.06)	52.17 (3.82–76.56)	42.50 (3.04–75.28)	22.75 (0.03–77.76)
Ruminococcaceae	15.74 (8.83–20.91)	5.78 (1.44–20.52)	9.31 (1.27–26.26)	13.90 (3.79–25.85)
Lachnospiraceae	9.94 (2.79–17.24)	10.03 (3.52–16.04)	5.14 (1.15–17.19)	7.09 (2.07–20.86)
Porphyromonadaceae	9.40.(2.75–16.99)	7.24 (0.05–16.08)	6.22 (2.54–22.57)	8.18 (2.04–30.51)
Rikenellaceae	5.84 (1.36–14.05)	2.77 (0.00–9.51)	4.13 (0.09–14.68)	5.27 (0.02–21.57)
Oscillospiraceae	3.43 (0.56–16.35)	1.10 (0.11–4.29)	1.12 (0.04–8.46)	1.13 (0.03–11.84)
Veillonellaceae	1.88 (0.01–5.51)	2.99 (0.00–7.62)	1.24 (0.01–9.74)	2.14 (0.39–8.93)
Eubacteriaceae	1.45 (0.23–4.39)	1.23 (0.05–12.16)	0.70 (0.04–4.11)	0.90 (0.06–3.90)
Prevotellaceae	1.13 (0.00–47.46)	0.03 (0.01–13.93)	0.08 (0.00–63.35)	3.09 (0.01–69.12)
Sutterellaceae	0.52 (0.00–12.11)	1.02 (0.00–33.65)	0.96 (0.00–7.20)	0.68 (0.00–3.52)
Clostridiaceae	0.48 (0.01–4.79)	0.06 (0.00–0.79)	0.14 (0.01–9.78)	0.22 (0.01–4.99)
Desulfovibrionaceae	0.37 (0.00–2.31)	0.27 (0.03–0.90)	0.31 (0.00–1.68)	0.45 (0.00–3.39)
Hyphomicrobiaceae	0.37 (0.07–1.72)	0.44 (0.07–1.38)	0.41 (0.01–7.13)	0.56 (0.01–8.00)
Acidaminococcaceae	0.32 (0.00–3.87)	0.00 (0.00–5.54)	1.54 (0.00–29.46)	0.002 (0.00–9.92)
Bifidobacteriaceae	**0.30 (0.02–1.58)***,**	**0.02 (0.00–6.65)***	**0.09 (0.00–3.86)***	**0.06 (0.00–2.43)****
Coriobacteriaceae	0.20 (0.04–1.74)	0.09 (0.01–0.72)	0.06 (0.01–1.36)	0.07 (0.00–0.50)
Lactobacillaceae	0.14 (0.00–1.19)	0.17 (0.00–1.36)	0.10 (0.00–5.27)	0.06 (0.00–1.14)
Pasteurellaceae	**0.12 (0.00–1.13)***	0.02 (0.00–0.11)	**0.003 (0.00–1.62)***,†	**0.025 (0.00–0.93)**†
Peptostreptococcaceae	0.11 (0.01–0.28)	0.18 (0.00–9.09)	0.06 (0.00–13.68)	0.20 (0.03–2.34)
Peptococcaceae	0.10 (0.00–0.84)	0.02 (0.00–0.53)	0.05 (0.00–0.92)	0.08 (0.00–0.58)
Erysipelotrichaceae	0.08 (0.02–0.33)	0.05 (0.00–0.81)	**0.06 (0.01–0.45)**††	**0.16 (0.03–3.34)**††
Clostridiales Family XIII, Incertae Sedis	0.06 (0.02–0.42)	0.06 (0.00–0.21)	0.21 (0.00–0.27)	0.06 (0.00–0.400)
Streptococcaceae	0.06 (0.00–1.10)	0.07 (0.00–0.43)	0.03 (0.00–1.55)	0.03 (0.00–0.35)
Enterobacteriaceae	0.05 (0.00–2.18)	0.17 (0.00–6.39)	0.05 (0.00–11.54)	0.11 (0.00–9.05)
Clostridiales Family XII, Incertae Sedis	0.02 (0.00–0.34)	0.05 (0.00–1.69)	0.02 (0.00–12.37)	0.03 (0.00–0.85)
Verrucomicrobiaceae	0.025 (0.00–2.39)	0.00 (0.00–0.07)	0.16 (0.00–16.51)	0.08 (0.00–3.06)
Graciibacteraceae	0.009 (0.00–0.03)	0.00 (0.00–0.55)	0.00 (0.00–0.10)	0.008 (0.00–0.25)
Bdellovibrionaceae	0.00 (0.00–1.37)	0.001 (0.00–0.37)	0.00 (0.00–3.27)	0.00 (0.00–2.83)
Comamonadaceae	0.00 (0.00–1.31)	0.00 (0.00–1.19)	0.002 (0.00–1.98)	0.002 (0.00–3.94)
Sphingobacteriaceae	0.00 (0.00–1.31)	0.00 (0.00–0.01)	0.00 (0.00–0.14)	0.00 (0.00–8.22)
Rhodospirillaceae	0.00 (0.00–0.96)	0.00 (0.00–5.33)	0.004 (0.00–2.41)	0.23 (0.00–3.66)
Spiroplasmataceae	0.00 (0.00–0.92)	0.00 (0.00–0.00)	0.00 (0.00–0.15)	0.00 (0.00–0.63)
Acholeplasmataceae	0.00 (0.00–0.42)	0.00 (0.00–0.00)	0.00 (0.00–1.54)	0.00 (0.00–3.99)
Synergistaceae	0.00 (0.00–0.28)	0.00 (0.00–0.13)	0.00 (0.00–0.72)	0.00 (0.00–0.03)
Anaeroplasmataceae	0.00 (0.00–0.21)	0.00 (0.00–0.00)	0.00 (0.00–0.80)	0.00 (0.00–0.11)
Victivallaceae	0.00 (0.00–0.20)	0.00 (0.00–0.00)	0.00 (0.00–0.94)	0.00 (0.00–0.35)
Oxalobacteraceae	0.00 (0.00–0.14)	0.00 (0.00–0.20)	0.00 (0.00–0.12)	0.00 (0.00–0.11)
Desulfobacteraceae	0.00 (0.00–0.05)	0.00 (0.00–0.00)	0.00 (0.00–0.35)	0.00 (0.00–0.12)
Flavobacteriaceae	0.00 (0.00–0.03)	0.00 (0.00–0.00)	0.00 (0.00–0.98)	0.00 (0.00–0.19)
Elusimicrobiaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.08)	0.00 (0.00–0.00)
Marinilabiaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–1.74)	0.00 (0.00–0.00)
Succinivibrionaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.63)	0.00 (0.00–0.08)
Puniceicoccaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.36)	0.00 (0.00–0.26

Data are presented as median and range (min–max) in %.
Kruskal-Wallis test was used for multiple comparisons between study groups. Statistically significant differences are indicated by $P < 0.05$ and marked in bold type. Significance: $^{*}P < 0.05$; $^{**}P < 0.01$, comparison HC to HIT, FH or FA; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, comparison between FH and FA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

The most abundant bacterial families found in all study groups were Bacteroidaceae (38.7%), Ruminococcaceae (11.2%), Lachnospiraceae (8.1%), Porphyromonadaceae (7.8%), Rikenellaceae (4.6%) and Veillonellaceae (2.1%). Moreover, the detailed analysis of bacterial families revealed significant differences in the proportions of Bifidobacteriaceae (class Actinobacteria; P = 0.050), Erysipelotrichaceae (class Erysipelotrichia; P = 0.018) and Pasteurellaceae (class Gammaproteobacteria; P = 0.031) between study groups.

On family level, the HC group harbored a significant higher proportion of Bifidobacteriaceae [0.30% (0.02 – 1.58%)] compared to the HIT [0.02% (0.00 – 6.65%); P = 0.036], FH [0.09% (0.00 – 3.86%; P = 0.027)] and FA [0.06% (0.00 – 2.43%); P = 0.007] group (*Fig. 3*, *Table 3*).

The percentage of Erysipelotrichaceae was significantly elevated in the FA group in comparison to FH group [0.16% (0.03 – 3.34%) versus 0.06% (0.01 – 0.45%); P = 0.012]. HC and FA group showed highest proportions of Pasteurellaceae [HC