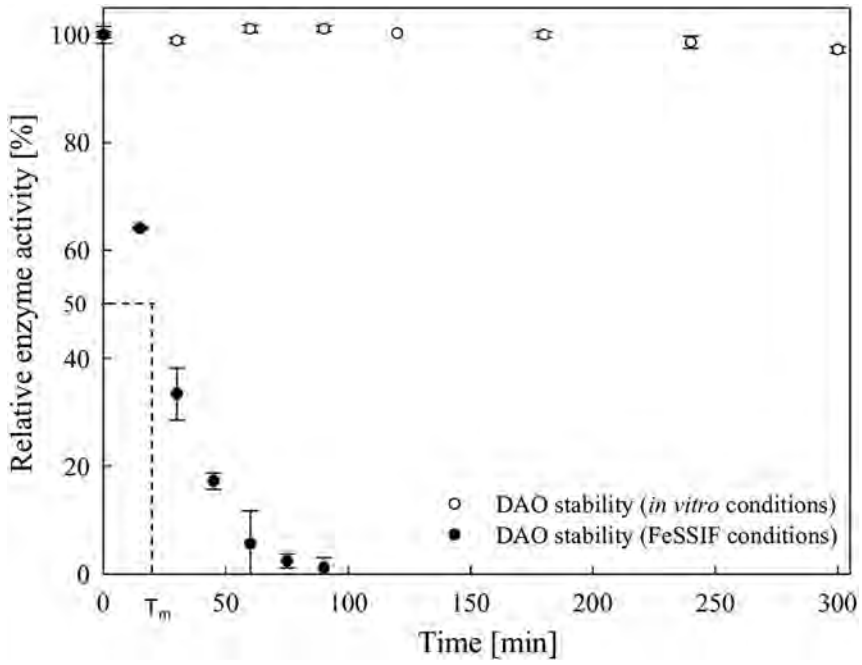

Figure 3–Porcine DAO stability under *in vitro* (20 mM sodium phosphate buffer, pH 8.0) and simulated intestinal conditions (fed state simulated intestinal fluid, pH 5.8) at 37 °C. Initially applied enzyme activity (100%) = 1.65 ± 0.08 nkat/mL.

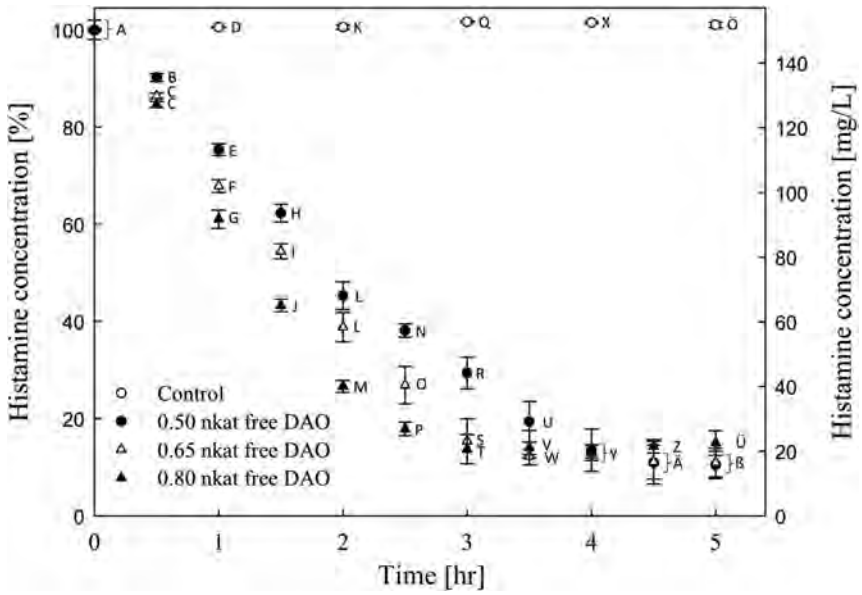

Figure 4–Histamine reduction using different activities of free DAO from porcine kidney (0.5, 0.65, and 0.8 nkat) in a food-relevant histamine concentration of 150 mg/L (20 mM sodium phosphate buffer; pH 8.0) at 37 °C. Letters (A to ß) indicate significance groups (Same letters = no significant differences; $P \leq 0.05$) within the used enzyme activities at the respective time points.

275 mL red wine (with 27 mg/L histamine), 150 g ripened cheese (with 350 mg/kg histamine), and 75 g sausage (with 200 mg/kg), resulting in a total of around 75 mg histamine in 500 mL (= 150 mg/L). According to our kinetic investigation, it has to be assumed that at a concentration of 150 mg/L porcine DAO is substrate inhibited by approximately 15% (Figure 2).

Using 0.5 nkat porcine DAO reduced the initial amount of histamine (0.75 mg) to 0.079 ± 0.002 mg, which equaled a relative histamine reduction of around 90% (Figure 4). However, the application of higher enzyme activities caused a faster histamine reduction but did not yield higher final reduction rates, which were stagnating between 85.0 ± 2.5% (0.8 nkat DAO) and 89.5 ± 2.8% (0.65 nkat DAO). Thereby, using 0.8 nkat DAO resulted in a significantly ($P \leq 0.05$) lower histamine reduction, which was most probably due to the increasing standard deviations at the lower histamine concentrations. Compressed manganese dioxide was added to the histamine conversion experiment to ensure that hydrogen peroxide formation was not responsible for a DAO inactivation. Because the overall histamine reduction did not improve with this addition, further experiments were performed without compressed manganese dioxide. The kinetics could be the cause of the incomplete histamine reduction. One reason might be that the inhibition constant K_i of the reaction product (imidazol-4-yl)acetaldehyde outweighed the Michaelis–Menten constant K_m of the substrate histamine. The amount of enzyme activity that is sufficient for the *in vitro* degradation of around 90% of the

histamine used (0.75 mg) within 5 hr was 0.5 nkat DAO in the downscaled experimental setup (5 mL). In conclusion, at least 0.1 nkat exogenous porcine DAO would be necessary for the reduction of 1 mL of a 150 mg/L histamine solution, whereby a complete reduction was not possible under the *in vitro* conditions tested. Using the partially purified free DAO preparation with a specific enzyme activity of 0.08 nkat/mg implies that at least 625 mg of the protein extract (equal to 50 nkat DAO) had to be used to counteract an exogenous histamine uptake of 75 mg.

3.5 Investigation of DAO capsules for the reduction of histamine

In a preliminary experiment, one DAO capsule was applied in 500 mL buffered histamine solution containing 75 mg histamine (150 mg/L) and no histamine reduction was measurable. Therefore, the following investigation of DAO capsules was also carried out in the downscaled 5 mL experimental setup as for the free DAO preparation. The DAO capsule consists of an outer cellulose shell that contains pig kidney extract beads, which are coated with shellac for acid resistance. The capsule build-up is displayed in the appendix (Figure S5). The DAO capsule preparation is supposed to withstand the acidic environment of the human stomach and eventually dissolves in the human intestine. A capsule was pretreated in SGF to test whether it could withstand the residence in gastric acid and whether it affects the efficiency of histamine reduction. The outer cellulose shell, used mainly for the practical administration of the DAO beads, dissolves quickly in SGF. The yellow capsule coating of the shellac-coated beads dissolved within 5 min in SGF, resulting in a near discoloration of the beads. Nevertheless, the beads maintained their overall structure and shape during the SGF treatment for at least 90 min. One SGF-treated and one non-SGF-treated DAO capsule were used in the histamine reduction experiments (Figure 5A), which were carried out according to the experimental setup described previously.

Because no DAO activity was detectable in the DAO capsule extract, the histamine reduction experiments using the free porcine DAO preparation (Figure 5B) were standardized regarding the protein content. The amount of protein in one capsule completely dissolved in buffer was 2.1 mg, determined according to Bradford (1976). However, the capsule manufacturer specified the encapsulated protein amount as 4.2 mg. Therefore, both protein amounts were applied to the buffered histamine solution to analyze the theoretical histamine degradation capacity of one DAO capsule. One DAO capsule decreased the amount of histamine used initially by 18.9 ± 2.3% ($n = 6$) within 5 hr, as shown in Figure 5A. Although the overall structure and shape of the DAO beads were retained during the treatment in SGF, the efficiency of the apparent histamine decrease was significantly ($P \leq 0.05$) reduced by around 7% to 12.1 ± 2.3% ($n = 6$). The experiment showed that the histamine content was decreased mainly within the first 30 min and stayed constant over the remaining 4.5 hr. However, the course of histamine decrease observed for the free DAO preparation (Figure 5B) was almost linear over the whole 5 hr. Within 5 hr, histamine was decreased by 23.7 ± 1.6% ($n = 3$) using 2.1 mg of free DAO (equal to 0.12 nkat in total). The usage of the double amount of free DAO resulted in a histamine conversion of 52.7 ± 0.8% ($n = 6$) (equal to 0.24 nkat in total). Free DAO was used together with one DAO capsule treated with SGF to exclude the possibility that DAO was inhibited by components of the capsule preparation (Figure S6). It was observed that histamine was steadily and similarly decreased within 5 hr, indicating that the DAO was not inhibited by components of the capsule preparation. Furthermore, an *in vitro* histamine reduction experiment was performed at 90 °C to evaluate whether the histamine reduction was due exclusively to the enzymatic degradation of histamine or to other reasons, such as adsorption of histamine to capsule components. An apparent decrease of histamine of 6 ± 0.5% was observed within 40 min using the DAO capsule at 90 °C (Figure S7). At 37 °C, the histamine was apparently decreased by 8.2 ± 0.1% within 40 min. By contrast, free DAO was inactivated at 90 °C, leading to no detectable histamine reduction within 40 min. The negative controls containing histamine without added DAO also showed no detectable histamine decrease within 40 min. Because the histamine content was reduced at 90 °C using the DAO capsule, enzymatic degradation of histamine was not the driving force leading to the histamine reduction in the *in vitro* experiments with DAO capsules.

The analysis of two different charges of DAO capsules showed differences regarding the apparent histamine decreases. One of the charges tested decreased the amount of histamine used (0.75 mg) by apparently 13.7 ± 1.3%, whereas the other charge tested reduced the amount of histamine by 3.9 ± 0.6%. This might be because the dietary supplement originates from natural sources and is, thereby, subjected to fluctuations among different charges (Figure S8).

3.6 Nonenzymatic reduction of histamine by DAO capsules

Although the applied histamine amount was partially reduced in the histamine reduction experiments using DAO capsules, no DAO activity was detected in the capsule preparation neither measuring the H_2O_2 formation (colorimetric DA-67 enzyme assay; Limit of detection (LOD): 1.75 μM) nor by determination of the (imidazol-4-yl)acetaldehyde product (RP-HPLC analysis; LOD: 25 μM). This nonenzymatic histamine reduction was further investigated by evaluating the capsule components. The components tested were microcrystalline cellulose, L-ascorbic acid, freshly ground shellac, polyvinyl pyrrolidone, and sodium carboxymethyl cellulose. Because the relative composition of the capsule preparation was not known, each component was used in an amount equal to the weight of the beads of one DAO capsule (206.3 mg). Only shellac was capable of reducing the histamine content in preliminary binding experiments (data not shown) and was further investigated. In addition, the medical product bentonite was selected for its histamine adsorption capability for comparison (Herr, 2010). The histamine reduction behavior of shellac and bentonite was tested in histamine-binding experiments and compared with one DAO capsule (Figure 6). Shellac apparently reduced the histamine content by 65.6 ± 1.5% within 5 hr.

Bentonite, consisting of much finer particles than shellac, apparently reduced the solubilized histamine content by 94.8 ± 3.6% within 5 hr (Figure 6). The DAO beads apparently decreased the histamine content by 18.9 ± 2.3%. Therefore, histamine was removed from the liquid phase through adsorption. Thus, it is suggested that the apparent reduction of histamine in the bioconversion experiments (Figure 5A) was caused by adsorption effects of the capsule content.

Incubating free DAO together with one DAO capsule (Figure S6) showed the expected (imidazol-4-yl)acetaldehyde peak in the RP-HPLC analysis for the used enzyme activity of free DAO and thereby verified that this product was not adsorbed by the DAO capsule. The slightly improved histamine reduction efficacy using the DAO capsule in combination with free DAO was explained by the enzymes' kinetics toward histamine

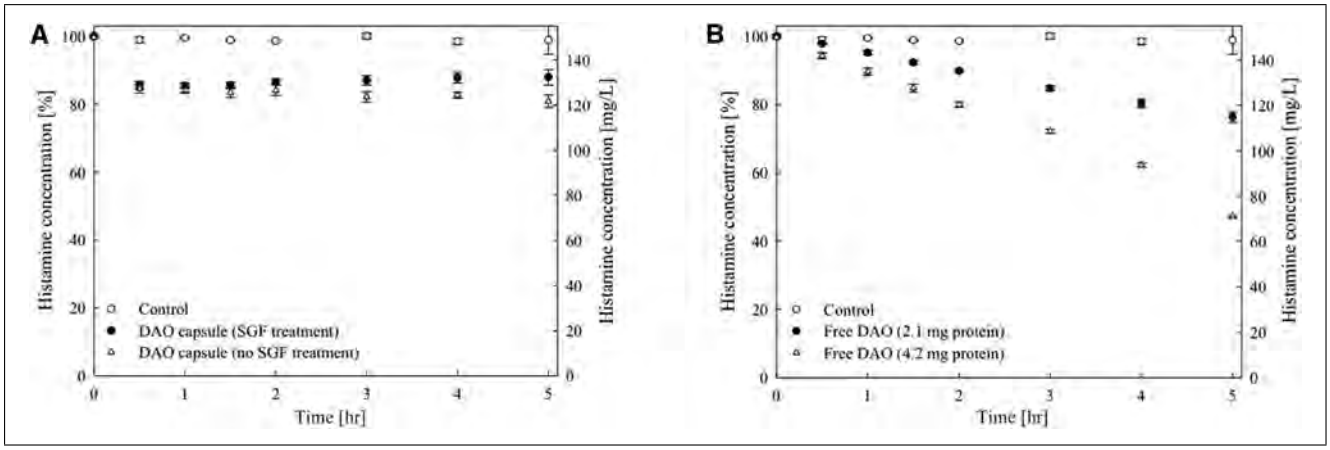

Figure 5–SGF-treated and SGF-untreated DAO capsules for *in vitro* histamine reduction (A). Equal protein amounts of free porcine DAO were used for comparison (B). Buffered histamine solution was incubated without capsules or free porcine DAO as a control.

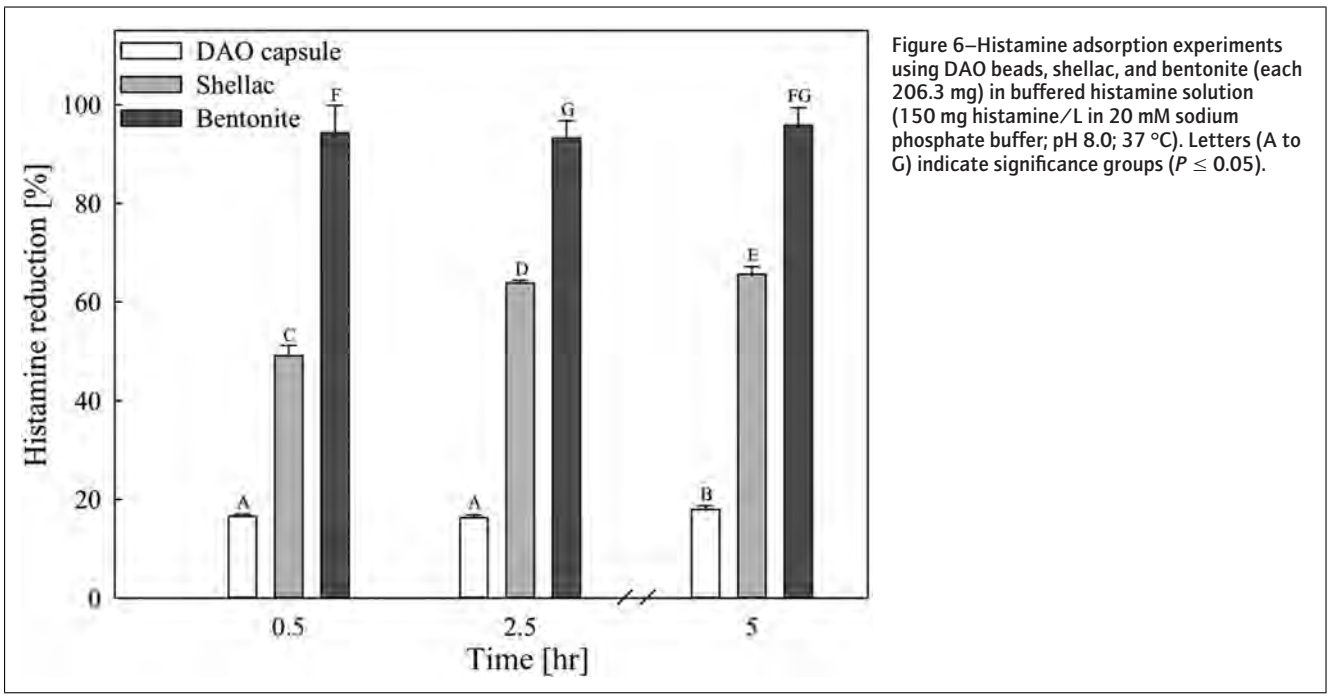

Figure 6–Histamine adsorption experiments using DAO beads, shellac, and bentonite (each 206.3 mg) in buffered histamine solution (150 mg histamine/L in 20 mM sodium phosphate buffer; pH 8.0; 37 °C). Letters (A to G) indicate significance groups ($P \leq 0.05$).

(Figure 2). By adsorption of histamine (substrate) at the beginning of the histamine reduction experiment, the free DAO was less substrate inhibited and therefore converted histamine with a higher efficiency.

3.7 Discussion

The enzyme DAO catalyzes the oxidative deamination of histamine to the reaction products (imidazol-4-yl)acetaldehyde, ammonia, and hydrogen peroxide (Schwelberger & Bodner, 1998). Our kinetic studies showed that porcine DAO is clearly substrate inhibited and probably also product inhibited (Figure 2 and 4) by histamine and (imidazol-4-yl)acetaldehyde, respectively. The relevant histamine concentration in foods seems to range from 10 to 200 mg histamine per liter or kilogram, respectively (Maintz & Novak, 2007). This is equal to a concentration of 0.09 to 1.8 mM. Because porcine DAO showed a substrate inhibition at histamine concentrations above approximately 0.5 mM (Figure 2), the enzymatic histamine conversion in foods contaminated with higher amounts of histamine will be lowered. This means that the decreasing DAO activity at higher histamine concentrations has to be compensated by usage of higher amounts of DAO. The supposed product inhibition of DAO is difficult to assess in the histamine conversion in foods because the product (imidazol-4-yl)acetaldehyde might react further to other compounds due to its chemical reactivity and, thus, not disturb the histamine conversion progress. An inhibition of DAO by the other reaction products hydrogen peroxide and ammonia does not seem to be relevant in concentrations encountered in our histamine reduction experiments (Bardsley, James, Crabbe, & Shindler, 1973).

The supplementation of exogenous porcine DAO to support the endogenous DAO in the human intestine is claimed to reduce histamine-associated physiological symptoms (Izquierdo-Casas et al., 2019; Komericki et al., 2011; Schnedl et al., 2019; Yacoub et al., 2018). However, we showed in our histamine reduction experiments that the DAO activity required for a satisfactory supplementation is considerably higher than expected. In the

study by Komericki et al. (2011), symptoms of histamine intolerance were analyzed and quantified after administering a defined amount of histamine (75 mg) to the participants. The oral administration of DAO capsules to reduce histamine-associated symptoms was then investigated. The research group found that the application of the DAO capsules in combination with tea containing histamine led to a statistically significant reduction of symptoms compared to the placebo approach ($P = 0.014$). In the study by Yacoub et al. (2018), DAO supplementation was found to be effective in relieving symptoms of urticaria. Recently, the studies by Izquierdo-Casas et al. (2019) and Schnedl et al. (2019) stated that the supplementation with DAO reduced the duration of migraine attacks and histamine intolerance related symptoms in general, respectively. This is not in accordance with our *in vitro* results and theoretical conclusions thereof. In our *in vitro* histamine reduction experiments (0.75 mg histamine), an apparent reduction of about 12% to 19% was observed within 5 hr using DAO capsules (Figure 5A). In our experiments, no autodegradation of histamine was observed, which is in accordance with the results of Dapkevicius et al. (2000) and Naila, Flint, Fletcher, Bremer, and Meerdink (2012). The latter found a histamine dihydrochloride solution to be stable at 30 °C for 1 month. Considering that the application of amounts of free porcine DAO equivalent to the protein content of one DAO capsule reduced the histamine (0.75 mg) by merely 24%, it can be concluded that this is not sufficient for a satisfactory histamine reduction in histamine-sensitive individuals. Our *in vitro* histamine reduction experiments using DAO capsules showed that histamine reduction was nonenzymatically caused by adsorption of histamine.

Using DAO to degrade histamine in a buffered system was evaluated by Dapkevicius et al. (2000) and Naila, Flint, Fletcher, Bremer, and Meerdink (2015) who investigated the histamine degradation efficacy for the production of fish silage and fish paste, respectively. Thereby, the usage of high DAO activities (180 nkat/g fish silage and 42 nkat/mL fish slurry) from porcine liver was useful for an extensive reduction of the histamine content in the fermented fish products. Naila (2012) also applied free DAO (42 nkat/mL) in a buffered system (0.5 M phosphate buffer; pH 6) and in tuna soup containing 500 ppm histamine and observed a complete histamine reduction within 10 hr for both experimental approaches. However, the focus of these histamine degradation experiments was on the production of histamine-reduced foods and animal feeds and not on the evaluation of DAOs' efficacy as a human food supplement.

In conclusion, our study showed that at least 50 nkat free porcine DAO were required to convert 75 mg histamine to (imidazol-4-yl)acetaldehyde in an *in vitro* test system. This resulted in a reduction of histamine by 90%. Furthermore, the enzyme showed weak stability under simulated intestinal conditions with a half-life period of around 19 min. Therefore, a lot more exogenous DAO would be required for efficient histamine degradation in the human intestine. The DAO capsule analyzed did not show DAO activity. Thus, a more suitable DAO with much higher activity and stability needs to be found.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Each author of the manuscript contributed to the formulation of the research questions and the design of the study. LK carried out the experimental work, analyzed the data, and wrote the manuscript, considering suggestions of IS and LF. All authors declared their approval of the final manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Mass spectrometry analysis of diamine oxidase (DAO) from pig kidney showing the sequence coverage for the DAO protein band (mascot enzyme none and tryptic digestion).

Figure S2. Mass spectrometry analysis of the DAO capsule Daosin showing the sequence coverage for DAO.

Figure S3. Native-PAGE (8%) analysis of the partially purified DAO preparation. Native protein marker (M) (21-720 kDa; Serva Electrophoresis GmbH).

Figure S4. Michaelis-Menten kinetics of porcine DAO linearized according to Hanes-Woolf; enzyme activity determined using the DA-67 assay with histamine (0.01 mM or 1.11 mg/L – 5 mM or 555.75 mg/L) as substrate at 37 °C in PIPES buffer (25 mM; pH 7.2).

Figure S5.DAO capsule consisting of an outer cellulose shell and the actual beads containing DAO.

Figure S6.*In vitro* histamine reduction using the commercially available DAO capsule preparation (SGF-treated) in combination with free DAO (4.2 mg) compared with free DAO (4.2 mg) without added DAO capsule.

Figure S7.(A/B). *In vitro* histamine reduction using the commercially available DAO capsule preparation (SGF-treated) (A) and free DAO from porcine kidney (B) in a food-relevant histamine concentration of 150 mg/L (equaled 0.75 mg histamine in total) in sodium phosphate buffer (20 mM; pH 8.0) at 90 °C and 37 °C.

Figure S8.*In vitro* histamine reduction using two different charges of the commercially available DAO capsule (charges 52901D and 71001D) in a food-relevant histamine concentration of 150 mg/L (equaled 0.75 mg histamine in total) in sodium phosphate buffer (20 mM; pH 8.0) at 37 °C.

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Biogenic Amines in Plant-Origin Foods: Are They Frequently Underestimated in Low-Histamine Diets?

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foods

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Review

Biogenic Amines in Plant-Origin Foods: Are they Frequently Underestimated in Low-Histamine Diets?

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Abstract: Low-histamine diets are currently used to reduce symptoms of histamine intolerance, a disorder in histamine homeostasis that increases plasma levels, mainly due to reduced diamine-oxidase (DAO) activity. These diets exclude foods, many of them of plant origin, which patients associate with the onset of the symptomatology. This study aimed to review the existing data on histamine and other biogenic amine contents in nonfermented plant-origin foods, as well as on their origin and evolution during the storage or culinary process. The only plant-origin products with significant levels of histamine were eggplant, spinach, tomato, and avocado, each showing a great variability in content. Putrescine has been found in practically all plant-origin foods, probably due to its physiological origin. The high contents of putrescine in certain products could also be related to the triggering of the symptomatology by enzymatic competition with histamine. Additionally, high spermidine contents found in some foods should also be taken into account in these diets, because it can also be metabolized by DAO, albeit with a lower affinity. It is recommended to consume plant-origin foods that are boiled or are of maximum freshness to reduce biogenic amine intake.

Keywords: histamine; putrescine; tyramine; cadaverine; biogenic amines; histamine intolerance; low-histamine diet; plant-origin foods; culinary process; storage conditions

1. Introduction

In recent years, various diets have been proposed for the treatment of histamine intolerance [1–8]. These diets, known as low- or free-histamine diets, usually exclude foods that patients associate with the onset of intolerance symptoms. Such foods tend to be rich in histamine, but some, surprisingly, are not usually regarded as sources of this amine.

As described in the literature and scientific reports issued by the European Food Safety Authority (EFSA) and a joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) committee, histamine intolerance (also called food histaminosis or food histamine sensitivity) is a disorder associated with increased plasma histamine levels and is recognized as clinically different from the more established histamine intoxication [9,10]. Although in both cases, histamine is the causative agent, the etiology of the disorders differs. Intoxication appears after the consumption of foods with unusually high histamine concentrations, while intolerance is due to a

deficiency in histamine metabolism, so that symptoms may be triggered even by the intake of low amounts [1,9–11].

Diamine oxidase (DAO) is the main enzyme responsible for the metabolism of histamine and other amines at the intestinal level, and impaired DAO activity is one of the main causes of histamine intolerance [1,12,13]. This enzymatic deficit may have its origins in genetic mutations. Different polymorphisms of a single nucleotide in the gene that encodes this enzyme (*AOC1* on chromosome 7) have been associated with lower DAO activity [14–16]. The deficit may also be due to acquired causes such as inflammatory bowel diseases that block the secretion of DAO [1,3,12], or to the inhibitory action of drugs, some of them with a very widespread use (e.g., acetylcysteine, clavulanic acid, metoclopramide, verapamil) [1,17]. Another enzyme involved in histamine metabolization is monoamine oxidase (MAO) [13]. Therefore, MAO inhibitor drugs, such as selegiline or rasagiline, could also favor the plasmatic accumulation of histamine and the onset of symptoms of histamine intolerance. In addition, the presence of other biogenic amines, mainly putrescine and cadaverine, may compromise the intestinal degradation of histamine by enzymatic competition with DAO [9].

The symptoms of histamine intolerance are numerous and highly variable, due to the effects and functions of histamine in multiple organs and systems of the body. They include gastrointestinal (abdominal pain, diarrhea, vomiting), dermatological (urticaria, dermatitis, or pruritus), respiratory (rhinitis, nasal congestion, and asthma), cardiovascular (hypotonia and arrhythmias), and neurological (headaches) symptoms, and it is common for more than one disorder to occur simultaneously [1,11,12]. Several clinical studies have shown that patients with a potential diagnosis of histamine intolerance or with a diagnosis of migraine, intestinal, or dermatological diseases (atopic dermatitis, eczema, or chronic urticaria) have a higher prevalence of DAO deficits compared to the control population [3,6,18–28].

In order to carry out a correct dietary treatment of histamine intolerance, it is necessary to know what foods may contain this amine and what factors influence its accumulation. Likewise, it is also important to consider the occurrence of other amines that are also metabolized by the DAO enzyme. In contrast to plant-origin foods, there is more available information on the contents of histamine and other amines in fish and fish derivatives and all types of fermented products (cheeses, sausages, sauerkraut, wines, beer), in which their presence is attributed to the aminogenic activity of spoilage microorganisms and also to fermentative microorganisms [9,10,29]. Therefore, the freshness of the food and the hygienic conditions of the raw materials and manufacturing processes, as well as the adequate selection of starter cultures without decarboxylase activity, are of vital importance to avoid or reduce the formation of these compounds [9,29–31].

Due to the information available on the contents of biogenic amines in nonfermented plant-origin foods being scarce, the aim of this study was to review the existing data on the contents of histamine and other biogenic amines in these types of products, as well as their origin and evolution during storage or cooking.

2. Methods

A selective search of scientific literature dealing with biogenic amine contents in nonfermented plant-origin foods, including vegetables, fruits, and cereals, was performed. The bibliographic search was carried out in the PubMed and Web of Science databases using the following keywords: "histamine", "biogenic amines", "tyramine", "putrescine", "cadaverine", "plant-origin food", "food samples", "storage", "cooking", "fruit", "vegetable", "legume", "cereal", "spinach", "eggplant", "tomato", "citrus", "modified atmosphere packaging", and "microbial decarboxylase activity". Original analytical studies, reviews, and table compilations of content in food were included. Articles published before 1990 were excluded from this review.

Apart from data obtained from the literature, data on the biogenic amine content of plant-origin foods from our own database of Spanish market products were also used. Specifically, histamine, tyramine, putrescine, and cadaverine contents of 25 types of vegetables, 19 fruits, and 8 cereals were included.

3. Content of Biogenic Amines in Plant-Origin Foods

In this section, the contents of biogenic amines (histamine, tyramine, putrescine, and cadaverine) in different plant-origin foods are reviewed, using our own database and data from studies published by other authors. A total of 20 studies reporting data on biogenic amine contents in such foods were found. Most provided data on putrescine contents (normally together with the polyamines spermine and spermidine, not dealt with in this section), and only a few included other amines, such as histamine, tyramine, and cadaverine.

3.1. Vegetables and Legumes

Table 1 shows the contents of biogenic amines in different types of vegetables and legumes (nonfermented).

The only products found to contain significant levels of histamine were eggplant, spinach, and tomato, each showing a great variability in content, both in samples from the same study and among different studies. Histamine values ranged from 4.2 to 100.6 mg/kg in eggplant, from 9.5 to 69.7 mg/kg in spinach, and from not detected to 17.1 mg/kg in tomato. In the case of asparagus, pumpkin, and chard, histamine was found in only a few samples and at very low levels (<2 mg/kg).

Histamine occurs naturally in certain foods [29,32], which explains why it was recorded in practically all samples of spinach, eggplant, and tomato. The variability observed may have been due to botanical variety, as reported by Kumar et al. [33] for eggplant. However, as occurs in foods of animal origin, the presence of high contents of histamine and other amines in plant-origin products could also be associated with microbial activity [29,32,34]. Lavizzari et al. [32] attributed the high contents of histamine in spinach to the activity of contaminating bacteria during storage, belonging mainly to the groups Enterobacteriaceae and Pseudomonadaceae. There is currently a need for more research to understand in more detail the origin of histamine in plant foods such as spinach, eggplant, and tomatoes.

Tyramine has been found in more foods than histamine, although in lower concentrations, in no case exceeding 10 mg/kg. It should be noted that histamine-containing foods also contained tyramine (eggplants, tomatoes, spinach, chard, and asparagus). Although there is very little information about the origin of tyramine in nonfermented vegetables, its presence seems to be associated with microbial aminogenic activity. The ability to form tyramine has been reported for bacteria of the genus *Enterococcus* isolated from plants and fruits, mainly *E. faecium*, *E. mundtii*, and *E. casseliflavus* [35].

Putrescine has been detected in all the studied vegetables and legumes, although its content varied greatly among foods and sometimes also within the same product. In most vegetables and legumes, the average values ranged from 1 to 25 mg/kg. However, some samples of green pepper, eggplant, sweet corn, green and purple beans, spinach, tomato ketchup, soybeans, and peas had strikingly high putrescine contents, in some cases exceeding 200 mg/kg (Table 1). The putrescine found in food can have a dual origin. In plant-origin foods, low contents of this amine generally have a physiological source, as it performs different functions in plants, as do the polyamines spermidine and spermine, ranging from the activation of organogenesis to protection against stress [34,36,37]. On the other hand, the presence of putrescine is also associated with the decarboxylase activity of different groups of spoilage bacteria, mainly Enterobacteriaceae and *Clostridium* spp. [36]. According to Kalač et al. [38], the high amounts of putrescine found in frozen peas are due to bacterial activity in the period between harvesting and freezing or during thawing. However, high putrescine contents cannot always be attributed to bacterial decarboxylase activity. Toro-Funes et al. [39] have suggested that the considerable levels of putrescine found in soybean sprouts arise from the germination process, as this amine is a plant growth factor. In general, based on the available information, and due to the great variability in the reported contents, it is difficult to establish to what degree the presence of putrescine in plant-origin products can be considered physiological or the result of bacterial activity.

Cadaverine, like tyramine, has been described in few vegetables and legumes and in relatively low concentrations, with average values that in no case exceeded 8 mg/kg. The values reported by Nishimura et al. [40] in onion (29 mg/kg) and tofu (18 mg/kg) were an exception.

3.2. Fruits and Nuts

Table 2 shows the content of biogenic amines in different types of fresh fruits, fruit juices, and nuts. There were fewer publications reporting amine data for this type of food than for vegetables and legumes. In general, the contents were low, putrescine being in many cases the only amine found (in addition to the polyamines spermidine and spermine).

Avocado and kiwi, and grapefruit, orange, and pineapple juices, are the only products in this category for which the presence of histamine has been reported, but not in all studies. The 23 mg/kg of histamine in avocado reported by Jarisch et al. [12] stands out, although no relevant information about its possible origin was provided. A study conducted by Preti et al. [41] concluded that the presence of histamine in grapefruit, orange, and pineapple juices is due to a lack of hygienic quality during processing or storage, since this amine is not found in the original fresh fruit.

Similarly, very few fruits contained tyramine, and levels have always been low (Table 2). Avocado and plum stand out for their content of this amine, although in no case has it exceeded 7 mg/kg.

Putrescine has been found in practically all the fruits and nuts, with the highest levels in orange, orange juice, mandarin, grapefruit, grapefruit juice, banana, passion fruit, and pistachio. The range of contents of this amine in citrus fruits and their juices has been very broad, varying from not detected to as high as 200 mg/kg. Suggested explanations for this variability have included different origins, cultivation, and transport and storage conditions [41–44]. As reported by Gonzalez-Aguilar et al. [45], the contents of putrescine in mandarin (flavedo) can be increased by a drop in temperature before harvesting and by damage of mechanical origin. Its presence in most of the samples, unaccompanied by high levels of other amines (related to bacterial activity), seemed to indicate that, with some exceptions, putrescine in fruits has a physiological origin. To confirm this, it would be necessary to carry out more studies analyzing the fruit at the moment of collection. The only fruits reported as having no putrescine were avocado and plum, although interestingly, these did contain histamine and tyramine.

The only fruits with a notable content of cadaverine were bananas and sunflower seeds, for which Nishimura et al. [40] reported average levels of 11 and 22 mg/kg, respectively, although these data were from the analysis of only two samples.

3.3. Cereals and Derivatives

Table 3 shows the contents of biogenic amines in cereals and some derivatives such as breakfast cereals, pasta, and bread. The quantitative information available on amines in cereals is very limited. In principle, these foods do not contain amines other than putrescine, which has a physiological origin [36]. The only standout source of putrescine is wheat germ, which, like soya bean sprouts, has a high rate of cell division, in which putrescine and polyamines play a significant role [36].

Table 1. Biogenic amine contents (mg/kg fresh weight) found in vegetables and legumes. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
Asparagus (wheat)	5	0.34 (0.62)	nd–1.42	0.69 (0.88)	nd–2.1	13.08 (2.81)	8.58–16.27	0.12 (0.19)	nd–0.43	#
Beans (green)	12	nd	-	2.46 (3.24)	nd–9.86	10.30 (8.61)	2.98–28.81	nd	-	#
	-	nd	-	nd	-	34.9 (6.2)	-	-	-	[46]
Beans (purple)	-	nd	-	nd	-	77.8 (7.7)	-	nd	-	[46]
Beans (yellow)	-	nd	-	nd	-	14.8 (1.4)	-	nd	-	[46]
Broccoli	4	-	-	-	-	9	7–10.5	-	-	[47]
	5	-	-	-	-	6.4 (2.9)	3.4–10.8	-	-	[42]
	10	-	-	-	-	5.7	-	-	-	[48]
	5	-	-	-	-	1.9	1.3–2.6	-	-	[49]
	2	-	-	-	-	-	8.37–20.53	0.1	-	[40]
Cabbage	3	-	-	-	-	-	-	-	-	[47]
	-	-	-	<0.5	-	16	-	-	-	[34]
	2	-	-	-	-	14.18	-	6.03	-	[40]
Cabbage (white)	10	-	-	-	-	6.6	-	-	-	[48]
	8	-	-	-	-	2.7	0.7–6.2	-	-	[49]
Cauliflower	3	nd	-	1.05 (0.91)	nd–1.71	3 (1.49)	1.61–4.58	0.16 (0.27)	nd–0.48	#
	3	-	-	-	-	-	3.1–4.5	-	-	[50]
	7	-	-	-	-	4.9	2.2–7.6	-	-	[47]
	5	-	-	-	-	5.3 (2.1)	3.3–8.9	-	-	[42]
	2	-	-	-	-	3.7	-	-	-	[40]
	-	-	-	<0.5	-	9	-	<0.5		[34]
Carrot	13	nd	-	nd	-	2.27 (2.20)	0.35–8.92	nd	-	#
	3	-	-	-	-	-	1.2–1.8	-	-	[50]
	4	-	-	-	-	2.8	2–3.9	-	-	[47]
	2	-	-	-	-	3.5	-	-	-	[51]
	6	-	-	-	-	1.5 (0.7)	0.7–2.7	-	-	[42]
	10	-	-	-	-	0.7	-	-	-	[48]
	4	-	-	-	-	12.1 (3.9)	7–18	-	-	[52]
	5	-	-	-	-	14.8	8–24.7	-	-	[49]
	-	-	-	1	-	5	-	-	-	[34]
	2	-	-	-	-	-	5.73–14.10	2.55	-	[40]
Celeriac	3	-	-	-	-	6.1	3.7–7.7	-	-	[47]
Chard	8	0.79 (0.41)	nd–1.33	1.90 (0.98)	0.74–3.48	6.38 (3.22)	2.4–11.94	0.13 (0.11)	nd–0.24	#
Courgette	41	nd	-	nd	-	7.94 (4.12)	2.74–24.81	0.32 (0.7)	nd–2.07	#
	-	-	-	2	-	4	-	-	-	[34]
Cucumber	10	nd	-	0.61 (0.89)	nd–2.33	5.42 (3.13)	1.32–10.62	nd	-	#
	3	-	-	-	-	3.2	-	-	-	[50]
	5	-	-	-	-	6.9 (1.4)	5.5–8.7	-	-	[42]
	10	-	-	-	-	8.7	-	-	-	[48]

Table 1. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
	5	-	-	-	-	13.1	2.1–28.8	-	-	[49]
	-	-	-	1	-	25	-	-	-	[34]
	2	-	-	-	-	9.87	-	2.96	-	[40]
Eggplant	23	39.42 (30.66)	4.17–100.6	0.60 (0.90)	nd–2.27	34.30 (6.98)	24.10–48.63	nd	-	#
	-	26	-	-	-	-	-	-	-	[12]
	2	-	-	-	-	18.35	-	5	-	[40]
Lettuce	4	nd	-	nd	-	2.85 (0.75)	2.20–3.90	nd	-	#
	3	-	-	-	-	-	3.3–4.8	-	-	[50]
	3	-	-	-	-	5.6 (1.3)	4.5–7.3	-	-	[42]
	10	-	-	-	-	7.9	-	-	-	[48]
	7	-	-	-	-	20.7	10.2–42.3	-	-	[49]
	2	-	-	-	-	6.87	-	3.27	-	[40]
Mushroom	11	nd	-	nd	-	1.29 (1.20)	0.02–3.65	0.10 (0.4)	nd–1.59	#
	10	-	-	-	-	11.7	-	-	-	[48]
	213	-	-	-	-	-	nd–156	-	-	[53]
Onion	4	nd	-	nd	-	nd	-	nd	-	#
	3	-	-	-	-		5.5–7.2	-	-	[50]
	10	-	-	-	-	0.5	-	-	-	[48]
	6	-	-	-	-	0.6	0.2–1	-	-	[49]
	-	-	-	3	-	2	-		-	[34]
	2	-	-	-	-	3.96	-	29.32	-	[40]
Pepper (green)	9	nd	-	nd	-	90.04 (41.65)	11.7–148.9	0.05 (0.14)	nd–0.41	#
	2	-	-	-	-	-	104–237	5.62	-	[40]
	5	-	-	-	-	70 (31)	13.2–96.9	-	-	[52]
Pepper (red)	8	nd	-	nd	-	2.42 (2.21)	0.59–5.35	nd	-	#
Potato	10	nd	-	0.58 (0.64)	nd–2.2	4.14 (3.06)	1.05–11.68	0.22 (0.54)	nd–1.75	#
	3	-	-	-	-	9.7	-	-	-	[50]
	3	-	-	-	-	17.6	-	-	-	[51]
	6	-	-	-	-	9.7 (2.1)	5.8–12.8	-	-	[42]
	2	-	-	-	-	-	0.1–22.4	-	-	[40]
	10	-	-	-	-	2.8	-	-	-	[48]
	6	-	-	-	-	7.2	1.1–10.5	-	-	[49]
	-	-	-	5	-	8	-	<0.5	-	[34]
Pumpkin	12	0.28 (0.54)	nd–1.90	nd	-	9.87 (6.19)	2.95–24.23	0.54 (0.76)	nd–2.15	#
Spinach	18	31.77 (17.02)	9.46–69.71	2.05 (0.83)	0.785–4.28	4.48 (2.46)	0.14–9.19	nd	-	#
	5	-	-	-	-	4.8	1.8–13.5	-	-	[49]
	-	16	-	6	-	6.0	-	1	-	[34]
	-	-	30–60	-	-	-	-	-	-	[12]
	-	37.5	-	-	-	-	-	-	-	[5]
	2	-	-	-	-	4.41	-	8.48	-	[40]
	-	61 (1.5)	-	nd	-	7.8 (0.1)	-	nd	-	[54]

Table 1. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
	32	-	-	-	-	12.9	nd–119	-	-	[52]
Sweet corn	5	nd	-	nd	-	38.44 (9.50)	30.5–119.2	0.18 (0.25)	nd–0.46	#
Tomato	53	2.51 (4.08)	nd–17.07	0.49 (0.92)	nd–6.38	16.48 (6.93)	6.29–35.55	0.50 (0.48)	nd–2.33	#
	3	-	-	-	-	-	9.3–122	-	-	[50]
	2	-	-	-	-	10.6	-	-	-	[51]
	5	-	-	-	-	10	5.3–20.7	-	-	[49]
	-	6	-	<0.5	-	23	-	<0.5	-	[34]
	-	22	-	-	-	-	-	-	-	[5]
	2	-	-	-	-	23.96	-	1.63	-	[40]
Tomato (concentrated)	19	-	-	-	-	25.9 (8.2)	7.9–41.1	-	-	[38]
Tomato (crushed)	3	1.22 (1.69)	0.24–3.17	0.14 (0.02)	0.12–0.16	9.66 (8.78)	4.5–19.80	0.18 (0.06)	0.12–0.23	#
	-	2	-	2	-	20	-	-	-	[34]
Tomato (ketchup)	3	0.37 (0.64)	nd–1.11	nd	-	1.07 (0.08)	1–1.15	nd	-	#
	24	-	-	-	-	52.5 (54.1)	nd–165	-	-	[38]
	-	22	-	-	-	-	-	-	-	[12]
Legumes and Derivatives										
Beans (white)	6	nd	-	nd	-	0.66 (0.64)	0.35–1.96	nd	-	#
	-	-	-	2	-	3	-	-	-	[34]
Beans (red kidney)	3	-	-	-	-	-	0.3–0.4	-	-	[50]
	5	-	-	-	-	-	nd–4	-	-	[52]
	-	-	-	3	-	1	-	-	-	[34]
Chickpeas	4	nd	-	nd	-	3.63 (2.49)	0.90–6.39	nd	-	#
	-	-	-	<0.50	-	2	-	<0.50	-	[34]
Lentils	7	nd	-	nd	-	8.19 (8.36)	1.96–21.81	nd	-	#
	5	-	-	-	-	-	nd–20.2	-	-	[52]
	-	-	-	-	-	3	-	-	-	[34]
Peanuts	7	nd	-	nd	-	0.87 (1.01)	nd–2.56	nd	-	#
Peas	9	nd	-	nd	-	34.28 (13.50)	8.74–54.44	nd	-	#
	10	-	-	-	-	17.3	-	-	-	[48]
	6	-	-	-	-	32.3	5.5–51.1	-	-	[49]
Peas (frozen)	14	-	-	-	-	46.3 (27)	11.7–107	-	-	[38]
Soybean, dried	3	-	-	-	-	-	1.6–6.5	-	-	[50]
	1	-	-	-	-	17	-	-	-	[47]
	2	-	-	-	-	41	-	-	-	[51]
	13	-	-	-	-	-	3.7–16.8	-	-	[55]
	4	-	-	-	-	30.9 (15.5)	16.3–57	-	-	[52]
	2	-	-	-	-	-	35.2–57.2	-	-	[40]
	5	-	-	-	-	17.1	6.4–24.2	-	-	[49]
Soybean milk	3	nd	-	nd	-	1.02 (0.73)	0.39–1.81	0.28 (0.24)	nd–0.42	[39]
	2	-	-	-	-	2.11	-	13.9	-	[40]

Table 1. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
Soybean sprouts	3	-	-	-	-	44.71 (3.21)	41.13–47.43	0.21 (0.18)	nd–0.33	[39]
Tofu	6	nd	-	nd	-	0.76 (0.55)	nd–1.49	0.67 (0.49)	nd–1.42	#
	4	-	-	-	-	nd	-	-	-	[49]
	19	-	-	-	-	2.6 (1.4)	nd-5	-	-	[56]
	2	-	-	-	-	1.76	-	18.4	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Table 2. Biogenic amine contents (mg/kg fresh weight) found in fruits and nuts. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Fruit and Fruit Products										
Apple	3	-	-	-	-	-	0.4–1.7	-	-	[50]
	2	-	-	-	-	nd	-	-	-	[51]
	2	-	-	-	-	1.5	-	nd	-	[40]
Apple juice	10	nd	-	0.67 (0.50)	nd–1.6	1.02 (0.35)	0.59–1.68	2.30 (1.53)	0.55–4.27	[41]
Avocado	5	nd	-	1.81 (2.06)	0.58–5.44	nd	-	nd	-	#
	-	23	-	-	-	-	-	-	-	[12]
	2	-	-	-	-	nd	-	nd	-	[40]
Banana	8	nd	-	0.53 (0.79)	nd–1.85	37.94 (8.32)	25.50–49.49	nd	-	#
	2	-	-	-	-	-	15.86–41.05	10.83	-	[40]
Cherry	5	nd	-	nd	-	3.08 (0.51)	2.35–3.46	nd	-	#
	2	-	-	-	-	4.67	-	nd	-	[40]
Grape	2	-	-	-	-	9.34	-	5.93	-	[40]
Grapefruit	2	nd	-	nd	-	55.55 (12.8)	46.52–64.57	nd	-	#
	2	-	-	-	-	51.1	-	nd	-	[40]
Grapefruit Juice	3	-	-	-	-	98.6	-	-	-	[50]
	10	0.31 (0.58)	nd–1.74	nd	-	10.08 (4.11)	7.17–20.8	1 (0.64)	0.38–2.28	[41]
Guava	21	-	-	-	-	1	0.4–1.8	-	-	[57]
Kiwi	13	nd	-	nd	-	2.49 (3.96)	0.5–15.57	nd	-	#
	2	-	-	-	-	1.06	-	nd	-	[40]
	-	1.9 (0.1)	-	nd	-	3.1 (0.1)	-	nd	-	[54]
Lemon	3	nd	-	nd	-	2.33 (2.02)	nd–3.67	nd	-	#
Mandarin	21	nd	-	0.94 (1.31)	nd–5.76	90.16 (36.6)	12.29–173.8	nd	-	#
	10	-	-	-	-	122 (44.2)	67.3–200	-	-	[42]

Table 2. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Fruit and Fruit Products										
Mango	21	-	-	-	-	0.9	nd–2.7	-	-	[57]
Orange	12	nd	-	nd	-	91.24 (41.7)	11.34–151.1	nd	-	#
	3	-	-	-	-	-	95.1–140	-	-	[50]
	2	-	-	-	-	117	-	-	-	[51]
	5	-	-	-	-	137 (11.3)	119–153	-	-	[42]
	2	-	-	-	-		54.62–119.82	2.04	-	[40]
Orange juice	3	nd	-	nd	-	45.51 (10.5)	37.35–57.3	nd	-	#
	3	-	-	-	-	85 (11.4)	76.6–100	-	-	[42]
	11	0.46 (0.41)	nd–1.32	nd	-	45.51 (8.35)	34.70–60.97	nd	-	[41]
Papaya	21	-	-	-	-	11	5.3–19.3	-	-	[57]
	2	-	-	-	-	4.67	-	nd	-	[40]
Passion fruit	21	-	-	-	-	17.9	6.5–40.5	-	-	[57]
Pear	3	-	-	-	-	-	23.6–24.2	-	-	[50]
	2	-	-	-	-	1.5	-	0.41	-	[40]
Peach	2	nd	-	nd	-	1.92 (0.14)	1.82–2.02	nd	-	#
	2	-	-	-	-	0.35	-	<0.10	-	[40]
Pineapple	6	nd	-	nd	-	4.20 (2.17)	1.39–7.96	nd	-	#
	2	-	-	-	-	4.05	-	3.07	-	[40]
	21	-	-	-	-	1.1	nd–2.5	-	-	[57]
Pineapple juice	12	2.44 (1.59)	nd–4.61	0.87 (0.86)	nd–1.93	1.79 (0.16)	1.53–1.98	1.21 (1.22)	nd–3.14	[41]
Plum	2	nd	-	4.02 (4.32)	0.96–7.07	nd	-	nd	-	#
Strawberry	9	nd	-	nd	-	3.77 (1.52)	2.04–6.42	nd	-	#
	2	-	-	-	-	0.97	-	4.29	-	[40]
Nuts										
Almonds	7	nd	-	nd	-	2.47 (1.24)	nd–4.36	nd	-	#
	2	-	-	-	-	4.32	-	5.57	-	[40]
Chestnuts	2	nd	-	nd	-	4.53 (3.40)	2.12–6.93	nd	-	#
	2	-	-	-	-	5.2	-	1.33	-	[40]
Hazelnuts	9	nd	-	0.49 (0.85)	nd–2.63	1.18 (1.09)	nd–3.19	nd	-	#
Nuts	6	nd	-	nd	-	5.64 (4.17)	2.82–13.79	nd	-	#
Pistachios	7	nd	-	nd	-	14.84 (14.0)	4.31–39.51	1.65 (4.37)	nd–11.58	#
	2	-	-	-	-	43	-	3.27	-	[40]
Sunflower seeds	2	nd	-	nd	-	0.50 (0.19)	0.36–0.63	nd	-	#
	2	-	-	-	-	3	-	22.58	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Table 3. Biogenic amine contents (mg/kg fresh weight) found in cereals and cereal-based products. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Barley	2	nd	-	nd	-	2.19 (1.55)	1.09–3.28	nd	-	#
Bread, white	2	nd	-	nd	-	nd	-	nd	-	#
	3	-	-	-	-	-	1.5–1.8	-	-	[50]
	10	-	-	-	-	1.1	-	-	-	[48]
	2	-	-	-	-	1.32	-	2.35	-	[40]
Bread, wholemeal	6	nd	-	nd	-	1.96 (1.45)	nd–4.32	nd	-	#
Cereal (corn, chocolate)	8	nd	-	nd	-	0.32 (0.44)	nd–0.93	nd	-	#
	10	-	-	-	-	-	2-2.2	-	-	[50]
Oats	2	nd	-	nd	-	0.67 (0.32)	0.44–0.89	nd	-	#
Pasta (wheat)	7	nd	-	nd	-	1.56 (1.65)	0.81–4.52	nd	-	#
Rice	2	nd	-	nd	-	2.4 (0.03)	2.38–2.42	nd	-	#
	2	-	-	-	-	<0.9	-	-	-	[51]
	2	-	-	-	-	1.2	-	-	-	[40]
	6	-	-	-	-	0.2	0.2–0.3	-	-	[49]
	10	-	-	-	-	0.2	-	-	-	[48]
Wheat germ	2	nd	-	nd	-	31.64 (0.35)	31.39–31.9	0.63 (0.08)	0.57–0.69	#
	2	-	-	-	-	62.1	-	-	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Putrescine contents in wholemeal bread were slightly higher than in bread made with refined flour. In white bread, low contents of cadaverine have also been reported, although only in one study, and from the analysis of two samples.

4. Evolution of Amine Contents during Storage and Cooking

The variability of amine contents observed among samples of the same product can be attributed mainly to conditions of production, transport, and storage [42].

The storage temperature is one of the most important factors in the formation of biogenic amines [11,29]. Refrigeration delays or reduces the aminogenic potential of microorganisms, although the formation of amines at refrigeration temperatures (4–10 °C) has been reported. The influence of the conservation temperature has been widely studied in foods such as meats, fish, and fermented products [29,30,58], but scarcely in plant-origin foods.

A study conducted by Simon-Sarkadi et al. [59] showed a clear increase in putrescine in different types of green leafy vegetables (lettuce, endives, Chinese cabbage, and radicchio) during six days of storage at 5 °C. The authors concluded that there was a positive correlation between putrescine contents and the hygienic state of these foods (total microorganism counts). Tyramine contents also showed a tendency to increase slightly. Histamine was present only in Chinese cabbage and in very low concentrations, remaining stable throughout the study period. In contrast, when Moret et al. [34] studied the effect of storage temperature on the amine content in various vegetables (parsley, zucchini, broccoli, and cucumber), no significant changes in histamine, tyramine, putrescine, and cadaverine were observed after three weeks of refrigeration.

Lavizzari et al. [32] also reported an increase in histamine in different spinach samples over 12–15 days of storage at 6 °C, noting that the relatively high pH of this vegetable favored the growth of Gram-negative bacteria, which could have been responsible for the formation of this amine during storage. The contents of tyramine and putrescine did not undergo significant changes under these storage conditions. It should be noted that in two of the five trials carried out in this study, histamine levels decreased in the last days of storage. The authors suggested that this histamine degradation could have been due to the action of bacteria with DAO activity, as well as the effect of the pH, which reached values above 8 [32]. Another study also recently reported the complete degradation of histamine in a spinach sample (61 mg/kg) after three weeks of storage at 4 °C [54].

Modified atmosphere packaging, together with low storage temperatures, is commonly used to extend the life of fresh vegetables and fruits. This type of packaging can influence the capacity of microorganisms to form amines [30,58,60]. Esti et al. [43] monitored the contents of amines during the ripening of cherries and apricots packaged in modified atmospheres and stored at 0 °C, and found that after 20 days of storage the contents of amines (mainly putrescine) had decreased by 20% compared to the initial value. Although the authors did not provide an explanation for this reduction, it could have been due to putrescine serving as a substrate for polyamine formation [36].

Another factor that can affect the content of biogenic amines in foods of plant origin, especially vegetables, is the culinary process. Again, the results reported in the literature were variable, depending on the type of cooking and the amine in question.

Latorre-Moratalla et al. [61] evaluated the effect of cooking spinach in water, with or without salt. The cooking process reduced the histamine content in all the samples by an average of 83% with respect to the raw product (after a correction for the dilution effect of the cooking). Analysis confirmed a transfer of histamine to the cooking water, which was not enhanced by the addition of salt. Likewise, Kumar et al. [33] observed the loss of 11–14% histamine in eggplants boiled at 100 °C for 10 min. Veciana et al. [62] also concluded that the putrescine content in certain vegetables (spinach, cauliflower, Swiss chard, potato, and green beans) is reduced by transfer to the cooking water. However, this heat treatment had no effect on the putrescine content in other vegetables such as pepper, pea, and asparagus. Eliassen et al. [42] also found no significant differences in putrescine levels among different types of raw and boiled vegetables (carrot, broccoli, cauliflower, and potato),

although they acknowledged that the low number of samples analyzed (two per food) was a limitation when trying to reach a conclusion.

Conversely, three recent studies have shown an increase in amine levels after a cooking process. According to Lo Scalzo et al. [63], boiling and grilling enhanced the putrescine content in a specific variety of eggplant by 55% and 32%, respectively. In the other two varieties of eggplant tested, the cooking had no effect. Similarly, Preti et al. [46] reported a significant increase in putrescine in green beans after boiling, whereas steaming did not modify the contents. According to the work performed by Chung et al. [64], frying brought about a 2.5- and 4-fold increase in histamine in carrots and seaweed, respectively. The authors attributed this increase to the loss of water caused by the high heat treatment. The same process had no effect on spinach and onions. However, it should be noted that in this study, the contents of histamine in all foods were well below 1 mg/kg, both before and after frying.

Amines are thermostable compounds, so in principle changes in contents can only be due to their transfer to the cooking water or by dilution or concentration effects of the culinary process, in which the food gains or loses water.

5. Plant-Origin Foods in Low-Histamine Diets

At present, the main strategy to prevent the onset of histamine intolerance symptoms is to follow a low-histamine diet. Its efficacy has been demonstrated in different clinical studies, which have always described an improvement or remission of gastrointestinal, dermatological, and neurological symptoms [3,6,18–20,22,24,27,65–67] if the diet was followed.

Current low-histamine diets exclude foods that patients associate with the onset of symptoms [1–8], such as blue fish and their preserves, and all kinds of fermented products (cheeses, sausages, wine, beer, sauerkraut, and fermented soy derivatives), all of which are susceptible to having high contents of histamine and other amines. A high number of nonfermented plant-origin foods are also excluded: The average contents of biogenic amines and polyamines in these foods are shown in Table 4. As can be seen, with the exception of spinach, eggplant, tomatoes, and avocado, for which high amounts of histamine have been described, the rest contained very little or no histamine, so a priori should not be responsible for triggering symptoms. However, some of them had relatively high contents of other biogenic amines and polyamines.

Table 4. Content of histamine and other biogenic amines (mg/kg fresh weight) in plant-origin foods excluded from different low-histamine diets [1–8]. Data obtained from own database and from different scientific studies [5,12,34,38–42,47–55,57].

Food Items	Histamine	Putrescine	Cadaverine	Tyramine	Spermidine	Spermine
Spinach [a]	9–70	nd–119	nd–9	1–10	14–53	nd–9
Eggplant [a]	4–101	24–49	nd–5	nd–2	2–12	nd–6
Tomato [a]	nd–17	5–122	nd–2	nd–6	2–16	nd–2
Ketchup [a]	nd–22	nd–165	nd	nd	nd–33	nd–12
Avocado [a]	nd–23	nd	nd	0.5–5	nd–7	2–8
Citrus (fresh and juices) [b]	nd–2	7–200	nd–2	nd–5	nd–12	nd–5
Mushroom [b]	nd	nd–156	nd	nd	9–155	nd–13
Banana [b]	nd	15–50	nd–10	nd–2	8–16	nd–3
Soybean or soybean sprouts [b]	nd	2–57	nd–0.3	nd	33–389	7–114
Nuts [b]	nd	nd–40	nd–23	nd–3	6–40	2–33
Pears [b]	-	2–25	nd–0.4	-	30–76	8–49
Lentils [b]	nd	nd–21	nd	nd	15–107	5–18
Chickpeas [b]	nd	1–6	nd–0.5	nd–0.5	15–85	4–32
Peanuts [c]	nd	nd–3	nd	nd	23–48	5–13
Kiwi [c]	nd–2	nd–15	nd	nd	3–6	nd–2
Papaya [c]	-	5–20	nd	-	4–8	nd–2
Strawberry [c]	nd	2–6	nd–4	nd	5–10	nd–2
Pineapple [c]	nd	nd–8	nd–3	nd	nd–3	nd–1
Plum [c]	nd	nd	nd	1–7	2–3	nd–4

Here, nd: not detected; -: values not reported by the studies; [a] plant-origin foods with histamine; [b] plant-origin foods without histamine but with high contents of other amines; [c] plant-origin foods with low levels of all amines.

Putrescine, cadaverine, and tyramine are all substrates of the DAO enzyme, so if present in high amounts they may increase the adverse effects of histamine by competing as rival substrates or for binding sites in the intestinal mucosa [1,9,68,69]. The high putrescine contents found in citrus fruits, mushrooms, soybeans, bananas, and nuts could thus explain why patients associate their consumption with the onset of histamine intolerance symptoms. However, it should be noted that some foods with similar or even much higher putrescine contents, such as green pepper, peas, or corn, are permitted in low-histamine diets (Table 1).

The polyamines spermidine and spermine can also be metabolized by DAO, albeit with a lower affinity [68,69], and therefore their presence should also be taken into account in this type of diet (Table 4). Thus, the exclusion of foods such as soybeans, mushrooms, lentils, chickpeas, peanuts, nuts, and pears may be justified by their high polyamine content.

Finally, the levels of biogenic amines and polyamines found in kiwi, papaya, strawberry, pineapple, and plum are too low to justify their exclusion. Some authors consider these foods, along with others such as milk, shellfish, and eggs, as endogenous histamine releasers, although by mechanisms still not well understood [1,11,70].

6. Conclusions

Biogenic amine data in nonfermented plant-origin foods from the different reviewed studies showed a great variability both within the same food item and among them. Putrescine was the most frequent biogenic amine found in fresh vegetables, legumes, fruits, and cereals, and only a limited number of products contained relevant levels of histamine (eggplant, spinach, tomato, and avocado). Tyramine and cadaverine were usually more scarcely found in plant-origin foods. Generally, low levels of histamine and putrescine may have a physiological origin. However, undesirable microbial enzymatic activity during production or storage may lead to the accumulation of high levels of these amines.

No single trend has emerged in the evolution of amine contents during refrigerated storage, which might be at least partly due to the different experimental designs of the studies. In some cases, refrigeration seems to have prevented the formation of certain amines, but this remains a hypothesis, as no study performed a comparative analysis of samples stored under refrigeration and at room temperature. The increase in the biogenic amine content during refrigerated storage reported by other authors may be attributed to bacterial activity. Additionally, some studies have observed an influence of culinary process on the biogenic amine content, mainly derived from the transfer of these compounds to the boiling water or by dilution or concentration effects of the applied treatment.

The exclusion of a high number of plant-origin foods from low-histamine diets cannot be accounted for by their histamine contents, but is more likely due to high levels of putrescine or spermidine. The plant-origin foods consumed by people with histamine intolerance should be of maximum freshness, since histamine and other amines may continue to form during refrigerated storage. The cooking of vegetables in water (boiling) is another relevant strategy for this population, since it can reduce the contents of histamine and other amines in the food.

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Diamine oxidase supplementation improves symptoms in patients with histamine intolerance

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Abstract Histamine intolerance (HIT) is thought to be caused by a disproportionate amount of histamine in the body. The enzyme diamine oxidase (DAO) is considered for the gastrointestinal degradation of histamine. For this open-label interventional pilot study, we identified 28 patients with HIT. For 4 weeks, they were instructed to take DAO capsules before meals. Then, throughout a follow-up period, they were instructed not to take the DAO. We used a questionnaire that included 22 symptoms, which were divided into 4 categories, as well as a symptom severity score. All symptoms improved significantly during the oral supplementation of DAO. During the follow-up period, without DAO supplementation, the symptoms sum scores increased again. The symptom intensity score was reduced for all symptoms. We have demonstrated, a significant reduction of every HIT-related symptom and its intensity due to DAO oral supplements.

The ClinicalTrials.gov identifier (NCT number) is NCT03298568.

Keywords Histamine · Diamine oxidase · Malabsorption · Gastrointestinal · Extra-intestinal

Introduction

Biogenic amines including histamine are produced by bacterial decarboxylation in food (Doeun et al., 2017). If the amount of ingested biogenic amines is high and/or their degradation is inhibited or disturbed in the body, then histamine is thought to cause multiple gastrointestinal (GI) symptoms. These may be accompanied by extra-intestinal symptoms including cardiovascular, respiratory and skin complaints (Reese et al., 2017). Symptoms of a disproportionate amount of histamine are thought to be caused by the reduced activity of the enzyme diamine oxidase (DAO).

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The clinical diagnosis of HIT is challenging. Although, patients with low serum DAO values, two or more GI symptoms described for HIT, and a reduction of complaints due to a histamine-reduced diet may be diagnosed with HIT. However, DAO is considered the main extracellular enzyme for the intestinal degradation of histamine and other biogenic amines (Elmore et al., 2007; Jones and Kearns, 2011). Therefore, the oral supplementation of commercially available DAO food supplements, prepared from pig kidney, has been suggested as a treatment of histamine intolerance (Smolinska et al., 2014). With this study, we aimed to demonstrate that the oral DAO supplementation improves symptoms, including GI, cardiovascular, respiratory and skin complaints in patients suffering from HIT.

Materials and methods

For this open-label interventional pilot study, we identified, in an analysis of outpatients' charts, 56 patients with recurring functional, nonspecific abdominal complaints and serum DAO values < 10 U/mL. At the time of diagnosis of HIT, all of these patients received written information on histamine intolerance and the histamine-reduced diet. A registered dietician helped develop an individually tailored diet to ensure nutritional adequacy. All of included patients maintained the histamine-reduced diet, although diet compliance was not separately evaluated for this study. Every patient had an easing of symptoms with the histamine-reduced diet. Although, they experienced a recurrence of functional nonspecific abdominal complaints, with serum DAO < 10 U/mL, at two separate evaluations, within 6 months before start of the study. Fifty-six patients were contacted by phone, 35 of them agreed to participate in the study. Thirty patients fulfilled the inclusion criteria with recurring functional nonspecific abdominal complaints and the screening DAO value < 10 U/mL, within 1 week before the study. Two patients were excluded during the study. One due to hospitalization and antibiotic therapy, and the other due to failing compliance. Therefore, 28 patients (male/female 7/21, median age 47.5 years, age range 19–72) completed the study according to the protocol and were included in the evaluation.

Study design

The patients were instructed not to change their diets or medication throughout the study period of 8 weeks. Symptoms, compliance of DAO capsule ingestion, and determinations of serum DAO and histamine in plasma were recorded at each visit, in 2-week intervals. For 4 weeks the patients were instructed to take DAOSIN® capsules, each containing 4.2 mg extracted pig kidney proteins with 0.3 mg DAO, before meals, up to three times per day. During the follow-up period of 4 weeks, the patients were instructed not to take DAO capsules. DAOSIN® was provided by Sciotec Diagnostic Technologies, Tulln in Austria (DAOSIN®).

We used a standardized questionnaire to assess the symptoms experienced by patients before and during the study period. The questionnaire was based on known symptoms and the four histamine receptors (Schnedl et al., 2019). Twenty-two symptoms were listed in four categories: GI, cardiovascular, respiratory and skin complaints. For each symptom, a severity score, from 0 (no symptoms) to 5 (very intense), was used. The patients were instructed to fill out this questionnaire at each visit. A radio extraction assay DAO Rea 100 (Sciotec Diagnostic Technologies, Tulln, Austria) was used for determination of DAO in the serum. The amount of histamine in the plasma was measured with an enzyme linked immunoassay, Histamin ELISA BA 10-1000 (Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany).

Evaluation of other food intolerance/malabsorption

All patients were examined and had neither lactose intolerance, fructose malabsorption, *Helicobacter pylori* infection nor celiac disease. We used hydrogen (H_2) breath tests for exclusion of lactose intolerance and fructose malabsorption (Gastrolyzer, Bedfont Scientific Inc., Kent, England). Either histologic evaluation of gastric mucosa or an enzyme-linked IgA immunosorbent assay (ELISA, Serion, Würzburg, Germany) showed absence of *Helicobacter pylori* infection. For screening of celiac disease antibodies against tissue transglutaminase were determined with anti-tTG IgA ELISA (Euro Diagnostica AB, Malmö, Sweden). The patients > 50 years old presented no pathology during a colonoscopy and the routine laboratory values were within normal range throughout the study.

Statistics

At every visit, during the study, the symptoms score was evaluated. For the assessment of the overall symptom severity, the score' sum was calculated. For detailed information on the distribution of symptoms, four categories—gastrointestinal, cardiovascular, respiratory and skin symptoms—were defined. Since the symptom score data is ordinally scaled, non-parametric tests were used for statistical calculations. The change in symptoms over time was calculated using the Friedman test. The Dunn's multiple comparison test was used as a post hoc test to identify the significance of the changes in symptoms at each visit. The Wilcoxon signed-rank test was employed to compare

the symptom score at the baseline (V1), after DAO ingestion (V3) and after the follow-up period (V5). It was also used to compare V1 and V5. Scatter-plots were used with medians and interquartile ranges (IQR), including minimal and maximal values (95% confidence interval). Calculations and graphs were done with GraphPad Prism Version 5.04.

Results and discussion

All 22 symptoms, including GI, cardiovascular, respiratory and skin complaints improved significantly (Table 1) during the oral supplementation of DAO from visit 1 to visit 3 (Wilcoxon $p < 0.0001$; Dunn $p < 0.001$). During the follow-up period from visit 3 to visit 5, the symptoms sum score increased (Wilcoxon $p = 0.0008$; Dunn $p < 0.01$), but, at visit 5, the symptoms sum score was still significantly lower than at visit 1 (Wilcoxon $p = 0.0004$) (Fig. 1).

Gastrointestinal symptoms

Evaluated GI symptoms, including abdominal pain, intestinal colic, bloating, diarrhea, constipation, nausea, belching, emesis, postprandial fullness and dysmenorrhea, significantly improved from visit 1 to visit 3 (Dunn $p < 0.001$; Wilcoxon $p < 0.0001$). During the follow-up period from visit 3 to visit 5, the symptoms sum score increased (Dunn $p < 0.05$; Wilcoxon $p = 0.0044$), but, at visit 5, the symptoms sum score was still significantly lower than visit 1 (Wilcoxon $p = 0.0026$). A significant reduction of symptoms with DAO ingestion was demonstrated using a sub-score, for the 5 most common and diagnostic GI symptoms of HIT: bloating, diarrhea, abdominal pain, belching and fullness. (Dunn $p < 0.001$; Wilcoxon $p < 0.0001$). During the 4 weeks of follow-up, a slight re-occurrence of symptoms was indicated (Wilcoxon $p = 0.0086$). Although, at visit 5, these symptoms were still significantly lower, compared to visit 1 (Wilcoxon $p = 0.0059$) (Fig. 2).

Other symptoms of histamine intolerance

Cardiovascular symptoms, including headache, vertigo, palpitations and collapse, significantly improved with DAO ingestion (Wilcoxon $p < 0.0001$). During the follow-up period from visit 3 to visit 5, the symptoms sum scores slightly increased (Wilcoxon $p = 0.028$), but, at visit 5, the symptoms were still significantly lower, when compared to visit 1 (Wilcoxon $p = 0.0033$). Respiratory symptoms, including rhinorrhea, nose congestion, sneezing and asthma, significantly improved from visit 1 to visit 3 (Dunn $p < 0.01$; Wilcoxon $p = 0.0142$). During the follow-up period from visit 3 to visit 5, the symptoms sum score increased (Dunn $p < 0.05$; Wilcoxon $p = 0.007$) and reached the sum score of visit 1 (Wilcoxon $p = 0.9099$) (Fig. 3). The symptoms of the skin—pruritus, urticaria, flush and swollen, reddened eyelids—significantly improved with DAO ingestion (Dunn $p < 0.01$; Wilcoxon $p = 0.001$). During the follow-up period from visit 3 to visit 5, the symptoms sum scores slightly increased (Wilcoxon $p = 0.0236$), but, at visit 5, the symptoms sum score, when compared to visit 1, was significantly lower (Wilcoxon $p = 0.0364$).

Symptom intensity

The symptom intensity, for every symptom a severity score from 0 (no symptoms) to 5 (very intense), indicated by patients was reduced for all symptoms—GI, cardiovascular, respiratory and skin complaints—due to the ingestion of DAO, as shown in Fig. 3. After the follow up period,

Table 1 Mean symptoms sum-score (± SD) of all symptoms, 10 GI symptoms, the 5 most diagnostic GI symptoms, 4 cardiovascular symptoms, 4 respiratory symptoms and 4 skin complaints during the study period of 8 weeks

Symptoms	4 weeks of DAO supplementation		4 weeks without DAO		
	Visit 1 Mean (± SD)	Visit 2 Mean (± SD)	Visit 3 Mean (± SD)	Visit 4 Mean (± SD)	Visit 5 Mean (± SD)
All symptoms	19.6 (± 9.9)	10.6 (± 7.7)	6.6 (± 6.4) $p < 0.0001$	10 (± 8.3)	12.5 (± 9) $p = 0.002$
GI	11.2 (± 7.5)	6.1 (± 5.2)	3.8 (± 4.4) $p < 0.0001$	6.5 (± 5.9)	7.1 (± 5.3) $p = 0.0058$
Diagnostic GI	8.4 (± 5.6)	4.8 (± 4.1)	3.1 (± 3.4) $p < 0.0001$	5 (± 5)	5.4 (± 4.3) $p = 0.0312$
Cardiovascular	3.6 (± 2.8)	1.9 (± 2.1)	1 (± 1.2) $p < 0.0001$	1.3 (± 1.7)	1.8 (± 2.2) $p = 0.0783$
Respiratory	2.4 (± 2.7)	1.6 (± 2)	1.2 (± 1.6) $p < 0.0001$	1.2 (± 1.7)	2.4 (± 2.6) $p = 0.0029$
Skin	2.4 (± 3.1)	1 (± 1.5)	0.5 (± 1) $p < 0.0001$	0.9 (± 1.6)	1.2 (± 2.2) $p = 0.1289$

The significance of the change in symptoms over time was calculated using the Friedman test

DAO diamine oxidase, *GI* Gastrointestinal

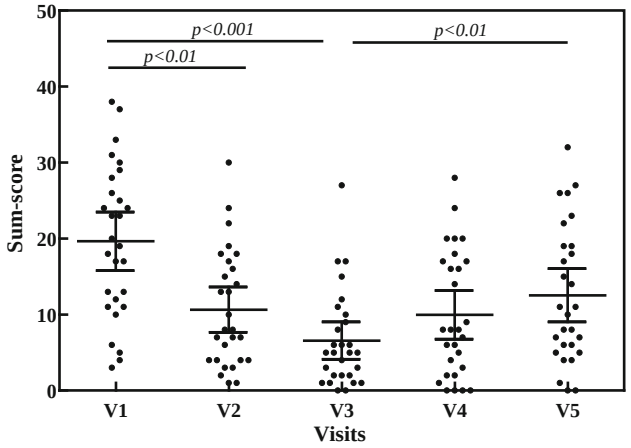

Fig. 1 Scatter-plot of symptoms sum-score of all 22 HIT-related symptoms throughout the study period of 8 weeks. Oral supplementation of DAO from visit 1 to visit 3 (V1 to V3); no oral DAO from visit 3 to visit 5 (V3 to V5)

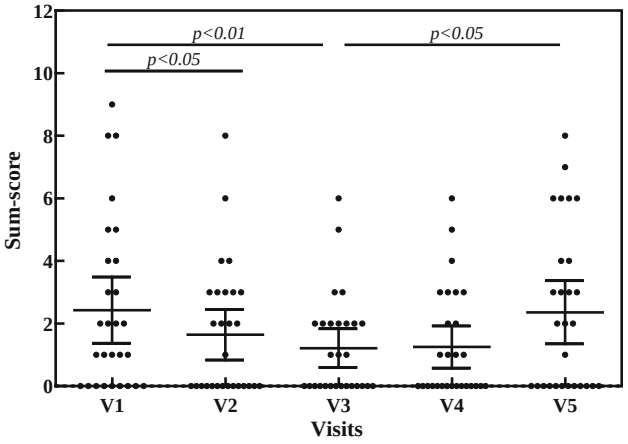

Fig. 3 Scatter-plot of symptoms sum-score of respiratory symptoms (rhinorrhea, nose congestion, sneezing and asthma) throughout the study period of 8 weeks. Oral supplementation of DAO from visit 1 to visit 3 (V1 to V3); no oral DAO from visit 3 to visit 5 (V3 to V5)

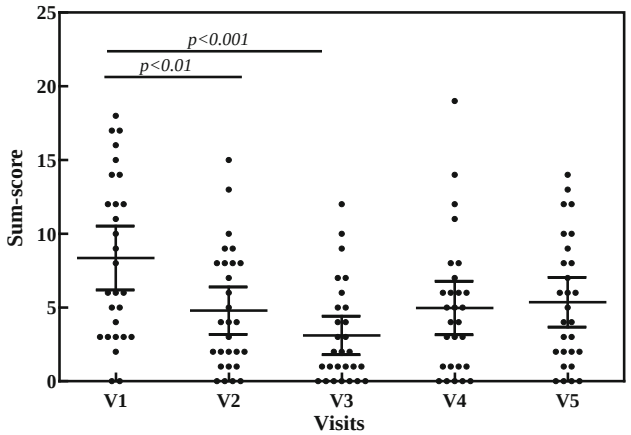

Fig. 2 Scatter-plot of symptoms sum-score of the most common and diagnostic 5 GI symptoms (bloating, postprandial fullness, abdominal pain, belching and diarrhea) for HIT throughout the study period of 8 weeks. Oral supplementation of DAO from visit 1 to visit 3 (V1 to V3); no oral DAO from visit 3 to visit 5 (V3 to V5)

none of the scores for symptom intensity returned to the intensity score of visit 1 (Fig. 4).

During DAO ingestion, 60.7% of patients showed slightly increased serum DAO values (Wilcoxon $p = 0.2918$), and DAO decreased again during the follow-up period (Wilcoxon $p = 0.346$) (Fig. 5). Histamine values, in plasma, remained unchanged during the study period.

Diamine oxidase and histamine intolerance

Biogenic amines like histamine, putrescine, cadaverine, and agmatine are produced by bacterial decarboxylation in foods. Usually, ingesting of low amounts of biogenic amines does not affect general wellbeing. The amount of biogenic amines in food is affected by several factors, including: the manufacturing process, hygiene of raw materials, microbial composition, and the duration of fermentation (Doeun et al., 2017). If ingested food contains a high amount of biogenic amines and/or their degradation is inhibited or disturbed, histamine accumulates in the body (Reese et al., 2017). DAO is, with its ability to degrade biogenic amines, considered the main enzyme in GI and extracellular degradation of histamine (Elmore et al., 2007; Jones and Kearns, 2011). It is synthesized by apical enterocytes located in the intestinal villi and is released from the mucosa for digestion and into the blood circulation (Wollin et al., 1988). DAO from the white pea (*Lathyrus sativus*) reportedly modulates histamine toxicity in vitro. A combination of DAO and catalase protected against histamine toxicity and prevented H_2O_2-induced damage on the human intestinal Caco-2 cell line occurring during histamine's oxidative deamination (Jumarie et al., 2017). An immune-regulatory influence of histamine within the gastrointestinal tract is known, but the effect of histamine on gut pathology, including inflammatory processes, is still poorly defined (Smolinska et al., 2014).

Diamine oxidase in serum

Recent findings speculated that low serum DAO values may be responsible for the symptoms of HIT (Kacik et al., 2018). Although, serum DAO values have not been established to reflect gastrointestinal DAO activity. However, the diagnosis of HIT, with the presence of two or more functional non-specific GI symptoms, may be supported with measurements of DAO values in serum (Reese et al., 2017). In this study, for diagnosis of HIT, besides

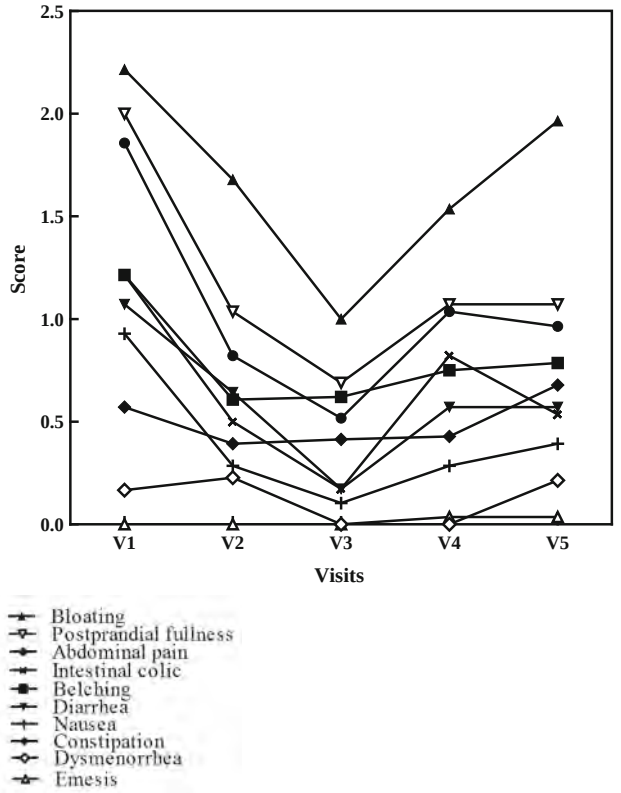

Fig. 4 Mean intensity of GI symptoms (abdominal pain, intestinal colic, bloating, diarrhea, constipation, nausea, belching, emesis, postprandial fullness and dysmenorrhea) throughout the study period of 8 weeks. Oral supplementation of DAO from visit 1 to visit 3 (V1 to V3); no oral DAO from visit 3 to visit 5 (V3 to V5)

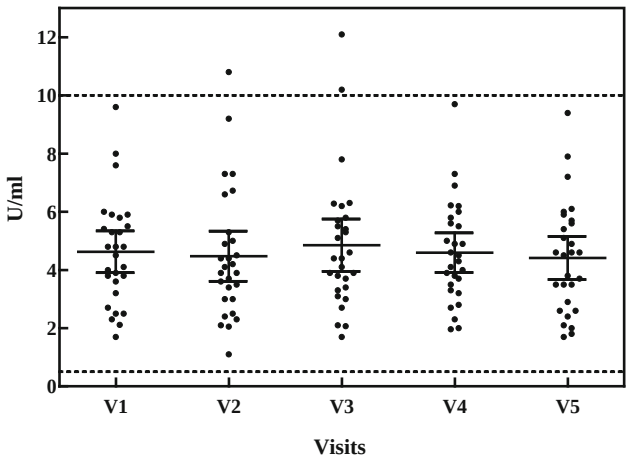

Fig. 5 Scatter-plot of serum DAO values throughout the study period of 8 weeks. Oral supplementation of DAO from visit 1 to visit 3 (V1 to V3); no oral DAO from visit 3 to visit 5 (V3 to V5). Normal range for DAO in serum 0.5–10 U/mL

functional nonspecific abdominal complaints, serum DAO values < 10 U/mL were used. Throughout the study, we have demonstrated slightly increasing serum DAO values, due to oral DAO supplementation.

A histamine-reduced diet was effectively shown to improve symptoms and potentially elevate serum DAO, after approximately 2 months. Depending on the compliance with a histamine-reduced diet, an improvement of HIT-related symptoms was demonstrated in nearly 80% of patients. Additionally, symptom improvement, combined with an increase of serum DAO values, occurred in more than 50% of patients (Lackner et al., 2019). Patients described GI symptoms to be the most prominent and severe of the HIT-related symptoms, along with a variability of symptom combinations (Doeun et al., 2017). It was reported that DAO capsules, taken in combination with histamine-containing tea, reduced symptoms of HIT (Komericki et al., 2011). Recently, oral DAO supplementation was reported as effective in patients with headaches. It significantly reduced the duration of migraine attacks (Izquierdo-Casas et al., 2019) and, in another study, caused symptom relief in patients with urticaria (Yacoub et al., 2018). We have demonstrated a significant reduction of all HIT-related symptoms with the oral supplementation of DAO. Before this study, 79% of the included patients complained of postprandial fullness and, 68% had bloating and abdominal pain. After 4 weeks of DAO ingestion, these symptoms were significantly reduced to 50% bloating, 43% postprandial fullness and 25% abdominal pain. Interestingly, we recognized that symptoms at the end of the study did not reach the same level of severity as at the beginning. We speculate that a slight recovery of the intestinal mucosa, due to the ingestion of DAO may be responsible. At visit 5, only the respiratory symptoms reached the same symptom sum scores as at the beginning of the study. This, we attributed to the fall season and its increased number of colds. However, a placebo effect, influencing the improvement of symptoms, in this study cannot be ruled out (Eisenbruch and Enck, 2015). Additional studies, including placebo-controlled randomized trials with high numbers of patients and long treatment periods, are needed to fully evaluate oral DAO supplementation.

Generally, a nutritionally adequate histamine-reduced diet can be developed, based on the individual's symptomatology, by reducing the amount of ingested histamine and biogenic amines. A histamine-reduced diet may present a challenge to HIT patients, because the composition of biogenic amines and levels of histamine in food and drinks are frequently unknown. In general, at least roughly 20 percent of patients with food intolerance/malabsorption cannot comply a comprehensive diet plan (Wilder-Smith et al., 2017). Therefore, we conducted this study in HIT patients and found that oral DAO supplementation helps reduce symptoms. We speculate that this may be due to its ability to degrade ingested intestinal histamine.

In conclusion, we have demonstrated that oral supplementation of the enzyme DAO before meals could help

improve HIT-related symptoms and symptom intensity in HIT.

Compliance with ethical standards

Conflict of interest M. Schenk, S. Lackner and H. Mangge declare no competing interests. W. J. Schnedl received speaking honoraria from Sciotec. F. Forster is employed by Sciotec. The authors are grateful for the free supply of DAOSIN® capsules from Sciotec. - Study monitoring and analysis of the data with BioTeSys GmbH was remunerated by Sciotec.

Ethical standard Written informed consent was obtained, the study was monitored, and the data was analyzed anonymously by BioTeSys GmbH, Esslingen (Germany). The study was done in accordance with the Helsinki Declaration of 1975, as revised in 2000 and 2008. The Ethical Committee of the Medical University in Graz approved this study (29-474 ex 16/17).

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Diamine Oxidase Supplementation in Chronic Spontaneous Urticaria: A Randomized, Double-Blind Placebo-Controlled Study. Mona-Rita Yacoub, Giuseppe A. Ramirez, Alvise Berti, Giuseppe Mercurio, Daniela Breda, Nicoletta Saporiti, Samuele Burastero, Lorenzo Dagna, Giselda Colombo. Int Arch Allergy Immunol. **2018**.

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Diamine Oxidase Supplementation in Chronic Spontaneous Urticaria: A Randomized, Double-Blind Placebo-Controlled Study

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Keywords
Diamine oxidase · Chronic spontaneous urticaria · Histamine · Histamine intolerance

Abstract
Introduction: Diamine oxidase (DAO) catabolizes and inactivates histamine, a key player in a wide range of invalidating conditions, such as migraine and chronic spontaneous urticaria (CSU). The highest expression of DAO occurs in the gastrointestinal tract, possibly to control the burden of histamine intake from food. ***Methods:*** Here, we tested the hypothesis that a 30-day oral supplementation with DAO (1 capsule b.i.d., 15 min before a meal) could reduce the severity of CSU as estimated by the 7-Day Urticaria Activity Score (UAS-7). The study was designed as a double-blind, placebo-controlled, crossover investigation of 22 patients with CSU incompletely controlled by first-line antihistamine therapy. ***Results:*** Twenty patients completed the study. Supplemental therapy with DAO caused a 3.8 ± 1.2 point mean ± SEM UAS-7 score reduction in patients with low serum DAO levels at time 0 (p = 0.041 compared to placebo). The degree of UAS-7 improvement was inversely correlated with the levels of basal DAO (p = 0.019). Patients receiving DAO supplementation were able to slightly reduce their daily antihistamine dose (p = 0.049). ***Conclusion:*** These data suggest that DAO may be involved in the pathogenic cascade of CSU and that DAO supplementation could be effective for symptom relief in patients with low DAO levels in serum.

Introduction

Chronic spontaneous urticaria (CSU) is a heterogeneous mast-cell-related disease characterized by recurrent flares of wheals and/or angioedema for more than 6 weeks, usually in the absence of clear offending triggers. CSU is highly prevalent in the general population and severely affects patients' quality of life [1]. Histamine lies downstream the pathogenic cascade that leads to the development of itch and wheals in CSU as well as in most

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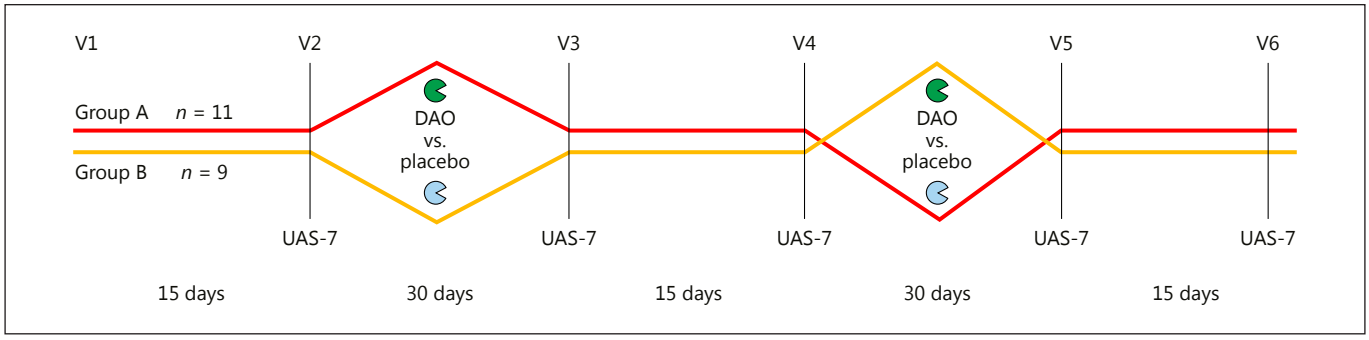

Fig. 1. Flowchart showing the study design and the duration of each study phase. Of the 22 patients recruited, 20 completed the study and were considered for the analysis. Eleven of them had been assigned to group A, 9 to group B. DAO, diamine oxidase; UAS-7, 7-Day Urticaria Activity Score; V1–6, study visits 1–6.

immediate-type hypersensitivity reactions, since it prompts vasodilation and increased vascular permeability [2]. Furthermore, histamine-related events have been implicated in the pathogenesis of other diseases such as atopic dermatitis and migraine [3]. Systemic and local levels of histamine are influenced by mast cell release, gastrointestinal absorption and catabolic efficiency. In particular, diamine oxidase (DAO) catabolizes histamine, thus neutralizing its biological effects [4, 5]. In mammals, DAO is mainly expressed at the level of the intestinal villi, in accordance with a peak enzymatic efficiency at pH 6.3 [6, 7]. Circulating levels of DAO may reflect DAO expression in the gastrointestinal tract [8]. Imbalances in the control of histamine levels due to inefficient DAO-related histamine clearance may lead to histamine accumulation and to the development of undesired histamine-related manifestations such as urticaria, accelerated gastrointestinal transit, or headache [2]. DAO supplementation has the potential to reverse these clinical manifestations [9], but data regarding its efficacy in CSU are lacking. We thus tested the hypothesis that circulating levels of DAO may reflect disease activity and that DAO supplementation may prompt symptom relief in patients with CSU.

Methods

We performed a double-blind, placebo-controlled, crossover study to assess the efficacy of a DAO supplement (Daosin®, AET-Pharma, Italy) in CSU. The study was approved by our local ethics committee. We enrolled 22 consecutive patients with CSU, lasting from an average of 6.8 years (0.25–17 years), unresponsive to a single daily dose of antihistamine and a 21-day histamine-free diet [1], diagnosed according to WAO guidelines at the San Raffaele Research Hospital, Milan, Italy [1]. Patients with concomitant gastrointestinal disorders, systemic autoimmune diseases, symptomatic IgE-mediated hypersensitivity to food allergens, previous severe anaphylaxis and/or elevated basal tryptase levels, other skin diseases, neoplasia, severe cardiopulmonary diseases or psychiatric illnesses, pregnant or lactating patients as well as patients with symptoms suggesting histamine intolerance other than urticaria were excluded. All enrolled patients were randomly allocated 1:1 to either group A or group B. The study lasted 105 days including (1) a first-entry, observational phase of 15 days; (2) a 30-day treatment phase in which group A received Daosin® 1 capsule 15 min before lunch and dinner, while group B received placebo; (3) a 15-day washout phase; (4) a 30-day treatment phase in which group A received placebo and group B the active treatment; (5) a final 15-day washout phase (Fig. 1). There were no specific dietary or drug restrictions during the study. Antihistamines in particular were administered, titrated, or tapered as per usual clinical practice. Serum levels of DAO were assessed at time 0 with a radioextraction assay using a commercial kit (DAOREA, AET Pharma, Italy) according to the manufacturer's instructions. The investigators were blinded with respect to basal DAO levels until the study was over. CSU severity as estimated by the 7-Day Urticaria Activity Score (UAS-7) and frequency of antihistamine use were measured on days 15, 45, 60, 90, and 105. Of 22 enrolled patients, 20 (11 in group A, 9 in group B) completed the study. Dropout was due to consent retreat in 1 case and change of residence in the other. After checking for normal distribution by the Kolmogorov-Smirnov test, UAS-7 variations among different study phases were compared between groups by employing the Student *t* test. Correlations between nonnormally distributed variables were analyzed by the Spearman test or Kruskall-Wallis test.

Results

Table 1 reports a summary of the patients' demographics. The mean ± standard error of mean (SEM) levels of tryptase and total serum IgE were 4.97 ± 0.66 μg/L (n = 14) and 170.01 ± 34.05 (n = 16), respectively. Five out of 20 patients had low (<10 U/mL) DAO levels at baseline

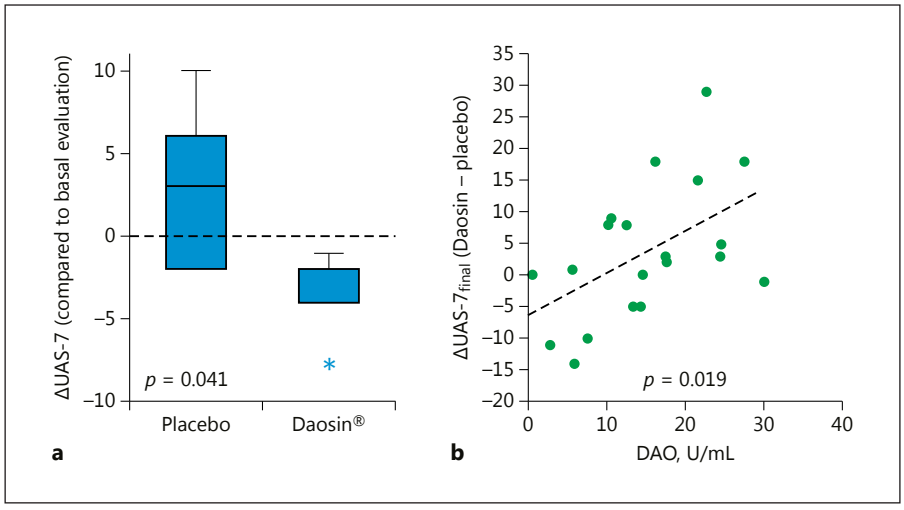

Fig. 2. a Reduction of 7-Day Urticaria Activity Score (UAS-7) with diamine oxidase (DAO) supplementation (Daosin®) and placebo in comparison to the basal UAS-7 in patients with low basal DAO. All patients with low basal DAO responded to the treatment. **b** Correlation between DAO levels in serum at time 0 and the net reduction of UAS-7 after treatment ($\Delta UAS\text{-}7_{final} = \Delta UAS\text{-}7_{Daosin} - \Delta UAS\text{-}7_{placebo}$).

Table 1. General demographics

Patient No.	Group	Gender	Age, years	Disease duration, years	Antithyroid immunity	Other autoantibodies
1	A	F	28	8	negative	no
2	A	F	24	1	negative	no
3	A	M	35	9	positive	no
4	A	F	19	0.25	negative	no
5	A	F	64	4	negative	no
6	A	F	59	0.5	positive	no
7	A	F	23	14	positive	no
8	A	F	41	6	positive	no
9	A	F	49	0.5	no	ANA
10	A	F	64	0.75	no	no
11	A	F	34	2.5	positive	no
12	B	F	59	6	no	no
13	B	M	60	0.16	no	no
14	B	F	48	1	no	no
15	B	F	70	17	no	no
16	B	M	55	0.33	no	ANA
17	B	M	35	0.5	no	no
18	B	F	45	0.5	no	no
19	B	F	59	0.5	positive	no
20	B	M	22	2	no	cold agglutinins

ANA, antinuclear antibodies.

[9]. When all patients' data were considered as a whole, there was no significant correlation between DAO basal levels and UAS-7 values at enrollment. The UAS-7 did not change significantly in either group during the treatment period, and no significant differences were detected in terms of disease severity between the placebo and the active group in both treatment phases. However, all patients with low basal DAO experienced a significant reduction of the UAS-7 score with Daosin®, when compared to placebo (mean ± SEM $\Delta UAS\text{-}7_{Daosin} = -3.8 \pm 1.2$ points vs. $\Delta UAS\text{-}7_{placebo} = 3.0 \pm 2.3$ points; $p = 0.041$; Fig. 2a). Basal levels of DAO correlated with the net effect of Daosin®, after adjusting for the placebo effect ($\Delta UAS\text{-}7_{final} = \Delta UAS\text{-}7_{Daosin} - \Delta UAS\text{-}7_{placebo}$; $p = 0.019$; Fig. 2b). The number of patients on antihistamines did not change significantly during DAO supplementation, when com-

Table 2. Antihistamine use throughout the study

	Baseline	DAO	Placebo	*p*
Patients regularly taking antihistamines, *n*	19/20	15/20	14/20	ns
Mean ± SEM daily dose of antihistamines, number of tablets	1.04±0.11	0.69±0.11	0.81±0.13	0.049

pared to the baseline evaluation or the phase in which patients took placebo. However, we observed a slight but significant ($p = 0.049$) reduction in the average daily dose of antihistamines when patients received DAO supplementation (Table 2). No other clinical features predicted a clinical response to DAO supplementation.

Discussion

In this small signal-seeking study, we aimed at finding clinical clues supporting a role of DAO as a disease modifier in CSU. Previous works suggest that imbalances in histamine degradation capacity may associate with the development of migraine and atopic dermatitis [3, 4], but little is known on the role of DAO in CSU. Taken together, our data suggest that DAO activity could be a cofactor in the development of CSU clinical manifestations and that DAO supplementation could contribute to a better disease control. Patients with low basal levels of DAO in serum may experience the most significant benefits. We acknowledge that the small sample size of our cohort as well as the lack of data regarding histamine levels and circulating levels of DAO after supplementation constitute potential limits of our study. In addition, by excluding patients with other potential symptoms of histamine intolerance in this trial setting, we might have sacrificed a wider understanding of the interactions between DAO and histamine in the real world to a clearer evidence about CSU. Larger studies are thus required to confirm our observations and possibly to extend the spectrum of DAO supplementation to other histamine-related manifestations.

Statement of Ethics

The authors declare that all patients involved in this study gave their written informed consent before being enrolled in the investigation. The study was approved by the ethics committee of the IRCCS Ospedale San Raffaele, Milan, Italy.

Disclosure Statement

The authors declare that there is no conflict of interest in connection with this work.

Funding Sources

The study was supported by AETPharma S.r.l., Inveruno (MI), Italy, which also provided the placebo/investigational product kits as well as the reagents for DAO measurement.

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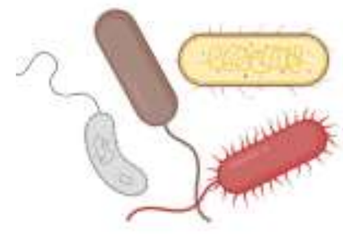
POSTERS AND AWARDS

Intestinal dysbiosis in patients with histamine intolerance



Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla and M. Carmen Vidal-Carou

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INTRODUCTION

Histamine intolerance is a disorder in histamine homeostasis, mainly provoked by a deficiency in the key enzyme responsible for histamine degradation at intestinal level, diamino oxidase (DAO), leading to a greater absorption of this amine, and the onset of the symptomatology[1,2]. This DAO deficiency could have a genetic, pharmacological or pathological origin[3]. Recently, it has been postulated that an alteration on the gut microbiota composition could also be a possible cause for DAO deficiency, though only a single study has challenged this hypothesis so far[4]. A major presence of histamine-secreting bacteria in the gut of histamine intolerance patients could also influence in the development of this intolerance[5].

The aim of this study was to characterize the composition of the intestinal microbiota of patients with histamine intolerance symptoms and to compare it with that of healthy individuals.

METHODOLOGY

A histamine intolerance group (HIT) was formed by 12 individuals from a centre specialized in histamine intolerance due to DAO deficiency and a control group with 14 healthy individuals. The analysis of the intestinal microbiota has been carried out from DNA isolated from stool samples (Qiagen) by sequencing of bacterial 16S rRNA genes (V3-V4 region) (MiSeq-Ilumina). The data analysis was performed by EzBioCloud Database.

RESULTS

As it can be seen in Figure 1, HIT group showed a significantly higher abundance of histamine-secreting bacteria in comparison with healthy group, including the genera *Staphylococcus, Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae* and the species *Clostridium perfringens* and *Enterococcus faecalis* ($p<0.05$).

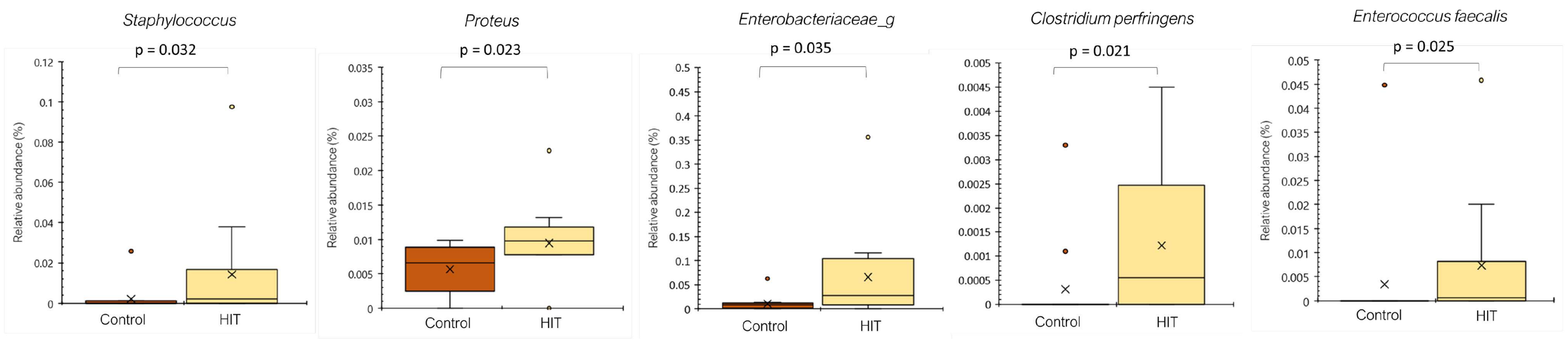

Figure 1. Relative abundance (%) of different histamine-secreting bacteria found in both study groups

A significantly low abundance of different bacteria associated with a healthy gut (*Ruminococcus*, *Faecalibacterium* and *Faecalibacterium prausnitzii*) was also found in HIT group ($p<0.05$) (Figure 2). *F. prausnitzii,* proposed as a marker of gut health is one of the most prevalent and abundant producers of butyrate in the human gut[6].

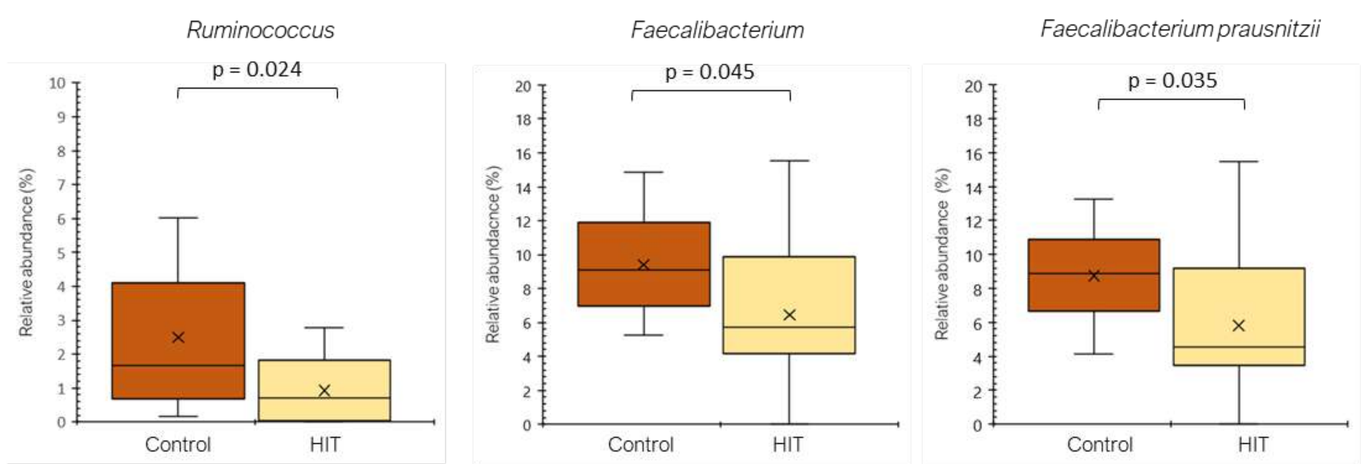

Figure 2. Relative abundance (%) of different healthy gut bacteria found in both study groups

CONCLUSIONS

A dysbiosis involving a greater abundance of histaminogenic bacteria would contribute to accumulate high levels of histamine in the gut, leading to a greater absorption of this amine in plasma and therefore, the appearance of adverse effects. The ability to degrade this histamine resulting from an intestinal dysbiosis would be easily overwhelmed in the individuals with DAO deficiency. Moreover, this dysbiosis could contribute to the mucosal inflammation which would ultimately affect the functionality of the DAO enzyme. An imbalance in the histamine-forming microbiota could account for another possible origin of histamine intolerance.

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Putrescine and cadaverine reduce the degradation rate of histamine by DAO enzyme

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INTRODUCTION

Diamine-oxidase (DAO) is the main enzyme responsible for the metabolism of histamine and other diamines at intestinal level. A DAO deficiency leads to an increase of histamine absorption from food and the appearance of symptoms associated with histamine intolerance (HI)[1]. Currently, the main strategy to avoid the symptoms of HI is to follow-up a low-histamine diet, which excludes foods that patients relate to the onset of symptoms. The diets reported in the literature usually excluded foods rich in histamine but also other foods without or with very low levels of this amine[2]. To explain this fact, it can be considered the hypothesis that the presence of other amines, such as putrescine and cadaverine, could cause a higher histamine absorption by competing for the DAO enzyme and the consequent appearance of the related symptoms (Figure 1).

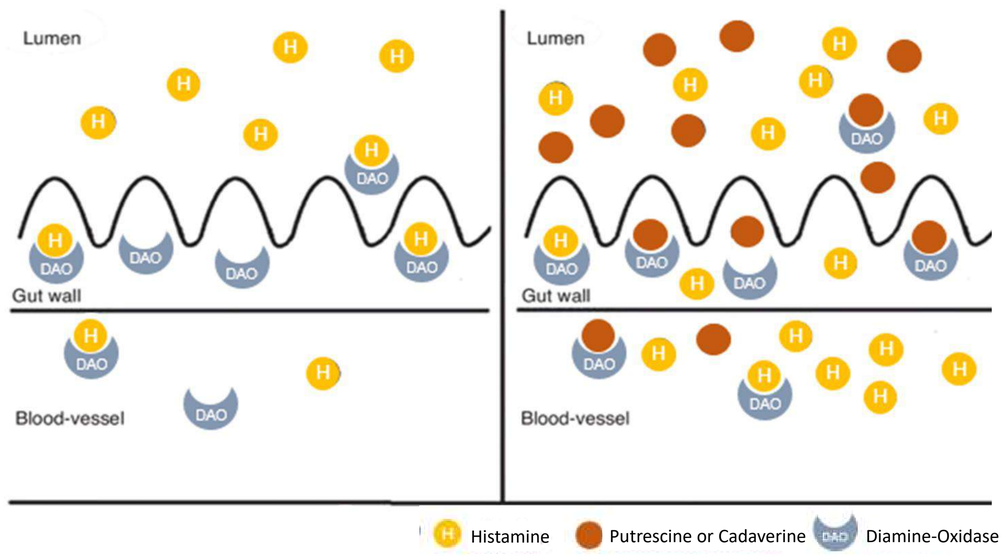


Figure 1. Diagram of histamine metabolism by the DAO enzyme at intestinal level when other amines are present.

OBJECTIVE

To assess *in vitro* if the presence of other amines, such as putrescine or cadaverine, interfere on the degradation of histamine by DAO enzyme

MATERIAL AND METHODS

The *in vitro* assay was performed with the addition of DAO enzyme in solutions with histamine alone, histamine together with putrescine or cadaverine in an equimolar proportion and at concentrations 4 times lower and higher than histamine. Histamine degradation was determined throughout 3 hours of enzymatic assay by UHPLC-FL[3] (Figure 2).

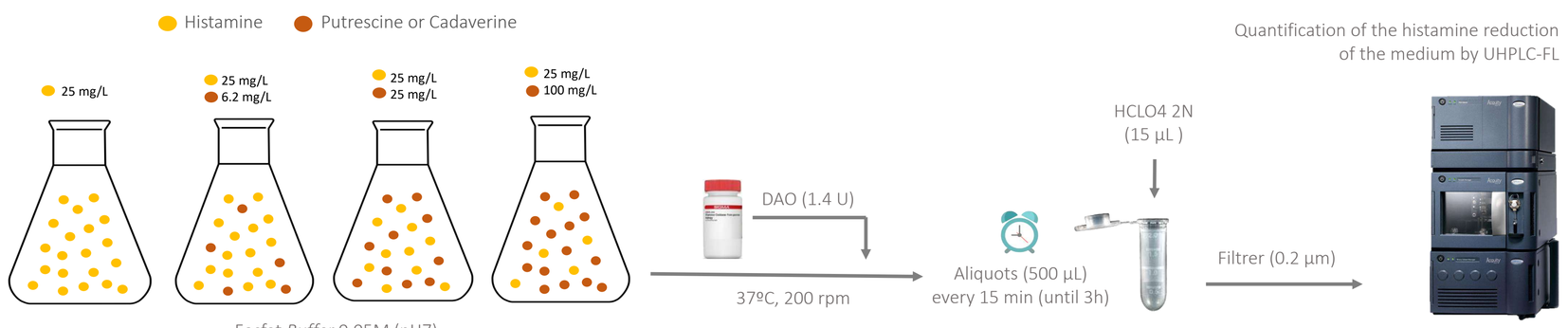


Figure 2. Diagram of the experimental assay for the *in vitro* kinetic study of histamine metabolism.

RESULTS

Figure 3 shows that histamine, putrescine and cadaverine were all of them substrates of the DAO enzyme. When histamine was alone it was totally reduced at 90 minutes of the assay, whereas putrescine and cadaverine required much more time to be totally degraded (until 180 minutes).

The presence of putrescine or cadaverine exerted an inhibitory effect on histamine degradation *in vitro* when they were at equal or higher levels than that of histamine (Figure 4A and 4B). Both putrescine and cadaverine delayed the degradation of histamine in 15 minutes when they were in an equimolar proportion and in 30 minutes when they were 4-fold higher. Concretely, when the concentration of putrescine or cadaverine was 4-fold higher than histamine, its percentage of degradation at 90 minutes of assay was 18% and 23% lower, respectively.

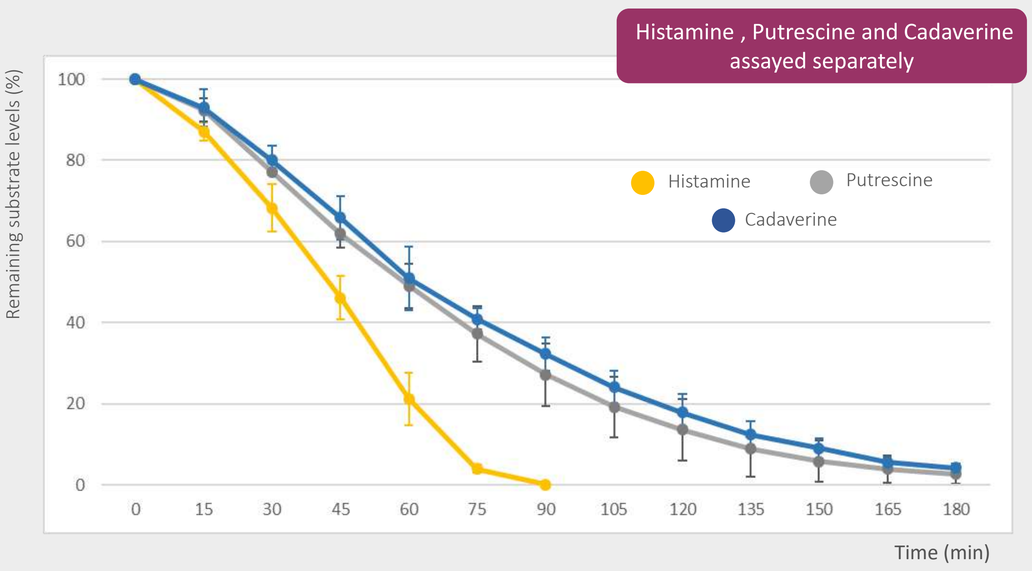
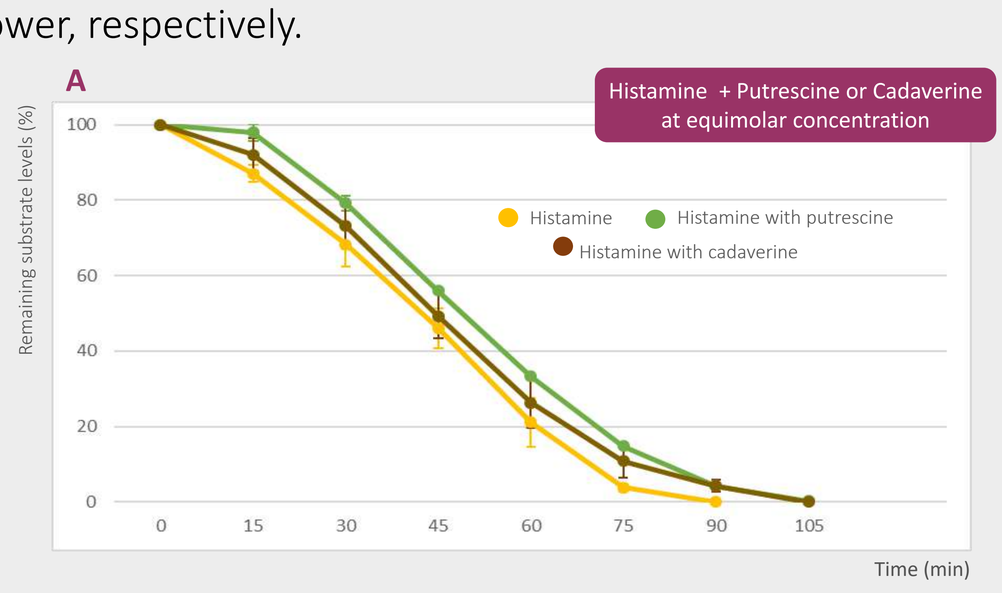


Figure 3. Remaining levels of histamine, putrescine and cadaverine throughout the time of the assay when they are alone with DAO enzyme in the solution.



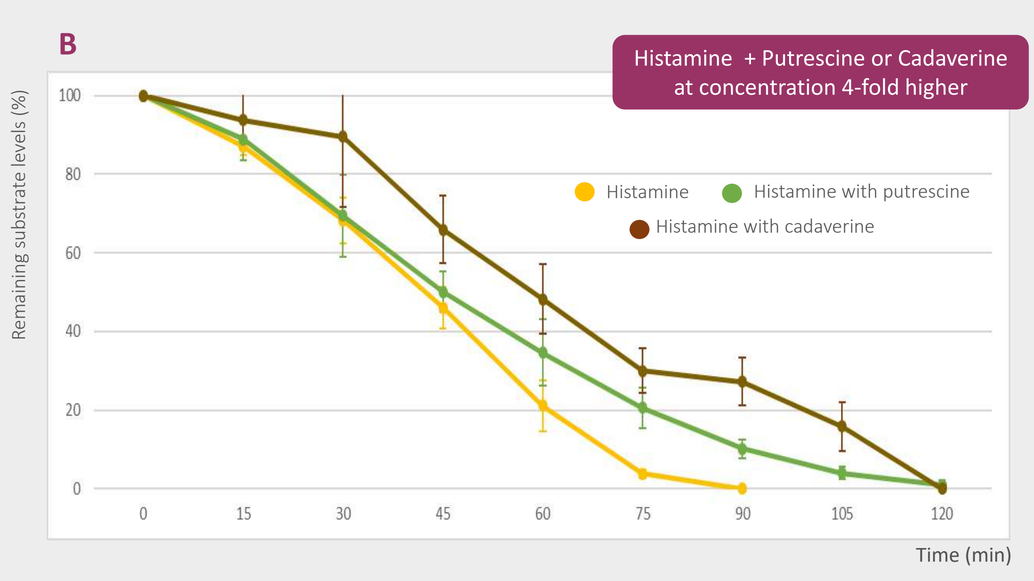


Figure 4. Remaining levels of histamine in its degradation by DAO enzyme, throughout the time of assay : A) histamine alone and in equimolar proportions between histamine and putrescine or cadaverine, B) histamine alone and 4-fold higher levels of putrescine or cadaverine that histamine levels.

CONCLUSIONS

The presence of putrescine and cadaverine reduces the degradation rate of histamine by DAO enzyme. Therefore, for the dietary management of HI, not only should be considered the foods with high levels of histamine but also those that could be an important source of these other amines, such as citrus, banana, sweet corn and green pepper.

References: [1]Maintz, et al.; 2007. Am. J. Clin. Nutr. 85: 1185-96. [2]Rosell-Camps, et al.; 2013. Rev. Esp. Enferm. Dig., 105: 201-6. [3]Comas-Basté, et al.; 2019. Anal. Bioanal. Chem.

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An enzymatic assay coupled to UHPLC-FL allows to screen for histamine-degrading activity in food matrices

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BACKGROUND

Diamine oxidase (DAO) is a homodimeric and ubiquitous enzyme found in microorganisms, plants and animals that catalyzes the oxidative deamination of the primary amino group of histamine[1]. In humans, intestinal DAO acts as a protective barrier against exogenous histamine, specially of food origin (Figure 1)[2,3]. A deficit of DAO activity can lead to the appearance of histamine intolerance, a clinical condition that may be treated by a low-histamine diet and oral DAO supplementation to enhance intestinal histamine degradation[4,5]. As sources of DAO, porcine kidneys and certain legume seedlings are suitable components for the formulation of a DAO supplement[5,6].

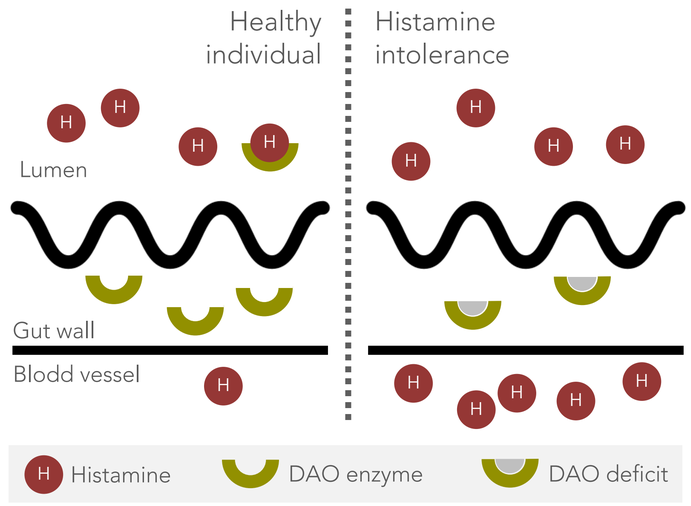

Figure 1. Intestinal degradation of histamine by DAO enzyme in healthy individuals (left) and in histamine intolerance (right).

OBJECTIVE

The aim of this work was to develop a rapid and reliable methodology for the *in vitro* determination of DAO activity in food matrices based on an enzymatic assay coupled to UHPLC-FL.

MATERIAL AND METHODS

The capacity of the DAO enzyme to degrade histamine was tested under controlled optimal conditions (37°C, pH 7.2) in a working solution with a defined initial concentration of histamine (Figure 2). The subsequent analysis of degraded histamine during the reaction time was performed by UHPLC-FL[7]. Specific DAO activity is expressed in mU/mg (nmol of degraded histamine per minute/mg of sample).

The applicability of the method was assayed with 13 different production batches of porcine kidney protein extract, 7 batches of lyophilized pea sprouts (*Pisum sativum*) and 6 commercialized DAO supplements available in the market.

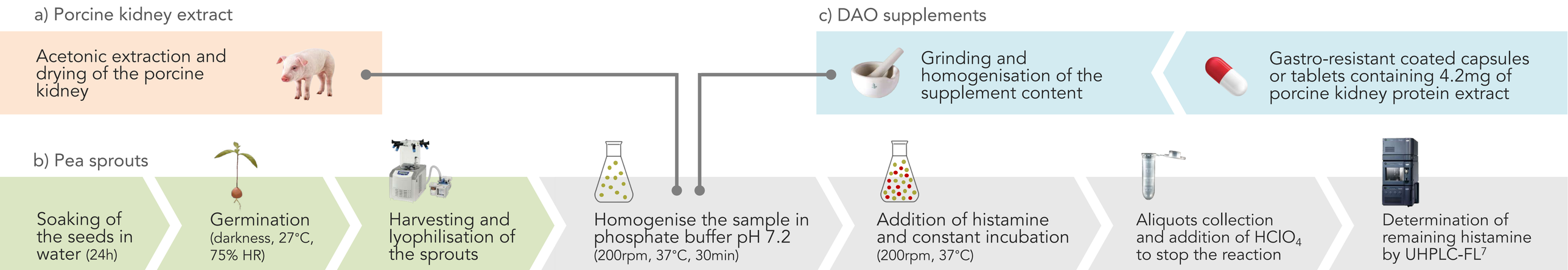

Figure 2. Schematic experimental procedure for *the in vitro* determination of DAO activity of porcine kidney extract (a), legume sprouts (b) and DAO supplements (c).

RESULTS

Method reliability

The proposed method showed satisfactory linearity (r=0.9998, p<0.001) and sensitivity (LOD=0.025 mU and LOQ=0.038 mU). Precision and recovery data are shown in table 1. A high specificity is one of the key attributes of this method due to the use of histamine as the substrate and the direct quantification of its degradation. Moreover, the lack of interference of catalase, an enzyme commonly found in plant and animal tissues, is another advantage in comparison with other published methods.

Table 1. Precision and recovery results for porcine kidney extracts and lyophilized pea sprouts.

	Precision		Recovery[c]			
	RSD (%)[a]	RSDH (%)[b]	Addition level I	Addition level II	Addition level III	Cochran's test C_{exp}[d]
Porcine kidney extract	2.76	3.45-4.60	100.54 (4.98)	102.69 (5.44)	99.14 (2.52)	0.41
Lyophilized pea sprouts	2.80	3.27-4.36	101.28 (0.90)	100.00 (0.76)	100.51 (2.61)	0.05

[a] Relative standard deviation (RSD) for seven determinations.
[b] Acceptable range for relative standard deviations according to the Horwitz equation for intra-laboratory studies (1/2 - 2/3 of the interlaboratory study calculate by the formula).
[c] Mean recovery percentages and standard deviation in parentheses for three addition levels corresponding to enzymatic activities of 0.5, 1.0 and 2.0 mU for porcine kidney extract and 1.0, 2.0 and 4.0 mU for lyophilized pea sprouts.
[d] Cochran's C variance outlier test, C_{tab} (6,2,0.05) = 0.8534.

Suitability of the method in real samples

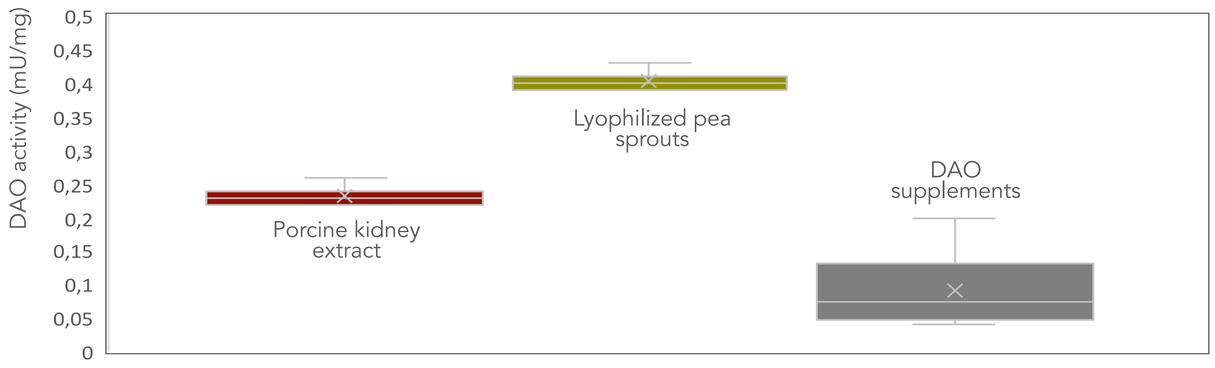

Figure 3. *In vitro* DAO activity of several production batches of porcine kidney protein extract, lyophilized pea sprouts and different commercial DAO supplements.

All analyzed products showed *in vitro* histamine-degrading capacity (Figure 3). Lyophilized pea sprouts were the most effective, with a mean activity of 0.40 (±0.01) mU/mg, compared to 0.23 (±0.01) mU/mg for porcine kidney protein extracts. These results are in good agreement with previously published data indicating a higher catalytic turnover rate for plant- than animal-derived DAO enzyme[6]. The enzymatic activity of the DAO supplements ranged widely from 0.04 to 0.20 mU/mg, despite all being formulated with the same amount of porcine kidney extract (4.2 mg).

CONCLUSIONS

The proposed method allowed the *in vitro* determination of DAO activity using histamine as the reaction substrate. This technique provided satisfactory experimental performance in terms of linearity, sensitivity, precision, and recovery, and its suitability was tested on different food matrices reported as sources of DAO. Due to the growing awareness of histamine intolerance, it is important to have effective methods for validating the DAO activity of supplements and foods of potential interest for the treatment of this disorder.

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HISTAMINE-DEGRADING CAPACITY OF LYOPHILISED LEGUME SPROUTS

O. Comas-Basté; J. Rabell-González; F. Pederzani; S. Sánchez-Pérez; N.C. Muñoz-Esparza; M.L. Latorre-Moratalla; M.T. Veciana-Nogués; & M.C. Vidal-Carou

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Introduction

Diamine oxidase (DAO), originally called histaminase, is the enzyme that performs the oxidative deamination of histamine[1]. In humans, intestinal DAO acts as a protective barrier towards exogenous histamine, specially from food[2]. A deficiency of DAO may lead to excess the normal plasmatic levels of histamine and the subsequent appearance of the symptomatology of histamine intolerance[3]. Certain legume sprouts have been described as a source of DAO, and thus could be a suitable component for the formulation of DAO supplements as a complementary treatment strategy for histamine intolerance[4].

Objectives

To perform a screening of the *in vitro* histamine-degrading capacity of commercial legumes and to study the stability of the enzymatic activity of the lyophilised product at different storage conditions.

Methods

Samples:

Lentils (*Lens culinaris*), chickpeas (*Cicer arietinum*), beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), peas (*Pisum sativum*), soy (*Glycine max*), alfalfa (*Medicago sativa*), grass pea (*Lathyrus sativus*), white lupin (*Lupinus albus*) and mung beans (*Vigna radiata*).

Methodology:

Lyophilised legume sprouts of the above-mentioned 10 different species were obtained in our laboratory. *In vitro* DAO activity was measured through an enzymatic assay coupled to UHPLC-FL analysis of remaining histamine (Figure 1). The stability of the DAO activity of the lyophilised product was assessed at three different storage conditions during 6 months: freezing (-20°C), refrigeration (2°C) and room temperature (±20°C).

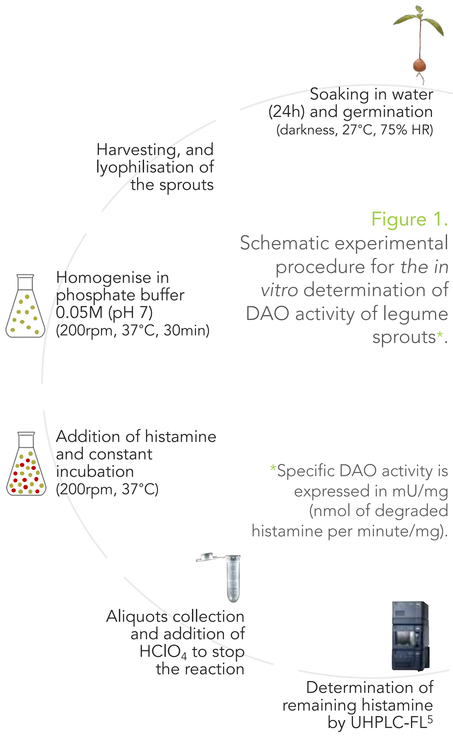

Figure 1. Schematic experimental procedure for *the in vitro* determination of DAO activity of legume sprouts*.

*Specific DAO activity is expressed in mU/mg (nmol of degraded histamine per minute/mg).

Results

Screening of histamine-degrading capacity:

Except for beans and mung beans, all analysed legume sprouts showed *in vitro* histamine-degrading capacity (Figure 2). Concretely, lyophilized pea and grass pea sprouts showed the greatest enzymatic capacity, with a mean DAO activity of 0.40 mU/mg.

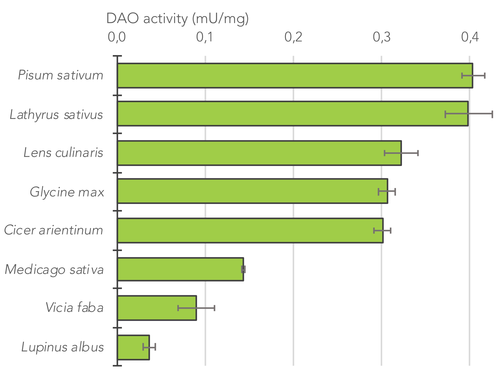

Figure 2. *In vitro* DAO activity of lyophilised legume sprouts germinated during 6 days in darkness.

Stability of the enzymatic activity:

Freezing storage of the lyophilized lentil sprouts kept the enzymatic activity intact for at least 6 months (Figure 3). On the contrary, the storage in refrigerator or at room temperature supposed a marked decrease in the enzymatic capacity of the product, with a mean loss of 47% (±0.02%) and 57% (±0.03%) of the initial DAO activity, respectively.

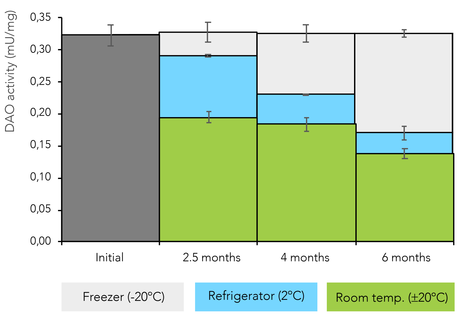

Figure 3. Evolution of the *in vitro* DAO activity of lyophilised lentil sprouts over 6 months of storage.

Conclusions

Lyophilised sprouts of certain legumes have been demonstrated to be a plant-origin source of DAO enzyme. This matrix could be a suitable component for the formulation of DAO supplements to enhance histamine degradation at intestinal level. Further studies are needed to select the optimal formulation of the final product to guarantee the stability of its enzymatic capacity.

Conflict of interest: Authors declare no conflict of interest.

References: [1]Schwelberger HG et al. J Neural Transm. 2013; [2]Kovacova-Hanuskova E et al. Allergol Immunopathol. 2015; [3]Comas-Basté O et al. J Pharm Biomed Anal. 2017; [4]Jumarie C et al. Appl Biochem Biotechnol. 2017; [5]Latorre-Moratalla ML et al. J Chromatograph A. 2009.

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WHAT ABOUT LEGUMES AS A PLANT SOURCE OF THE DAO ENZYME?

Comas-Basté, O., Sánchez-Pérez, S., Garza-Guajardo, R.I., Latorre-Moratalla, M.L., Veciana-Nogués, M.T., & Vidal-Carou, M.C.

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BACKGROUND

Exogenous diamine oxidase (DAO) enzyme has been recently postulated as a potential strategy for the treatment of histamine intolerance, a disorder in the homeostasis of histamine caused by a reduction of its intestinal degradation[1]. Dietary supplements based on gastro-resistant encapsulated porcine kidney protein extract are currently available on the market to reduce the symptomatology associated with this intolerance by enhancing the degradation of dietary histamine in the intestine[2]. Although with scarce scientific research, there are some references that have pointed out the potential of pea seedlings as a plant source of DAO enzyme[3-4]. If confirmed, peas and, potentially, other legumes could become an interesting alternative to porcine DAO enzyme to widen the target population and with advantages from a productive and sustainable perspective.

OBJECTIVE

To study the capacity of legumes and their sprouts to reduce histamine *in vitro* and evaluate the influence of different growing conditions (light exposure and sprouting duration) and treatments (lyophilisation) on this enzymatic activity.

MATERIAL AND METHODS

Samples were both raw pulses and sprouts of lentils (*Lens culinaris*) (brown, beluga and organic), chickpeas (*Cicer arietinum*), beans (*Phaseolus vulgaris*) (white, black, black-eyed, kidney and DOP *Ganxet*), broad beans (*Vicia faba*), peas (*Pisum sativum*) and mung beans (*Vigna radiata*). *In vitro* DAO activity was measured through an enzymatic assay and the subsequent analysis of degraded histamine by UHPLC-FL (Figure 1). Specific DAO activity is expressed in mU/mg (nmol of degraded histamine per minute/mg or g).

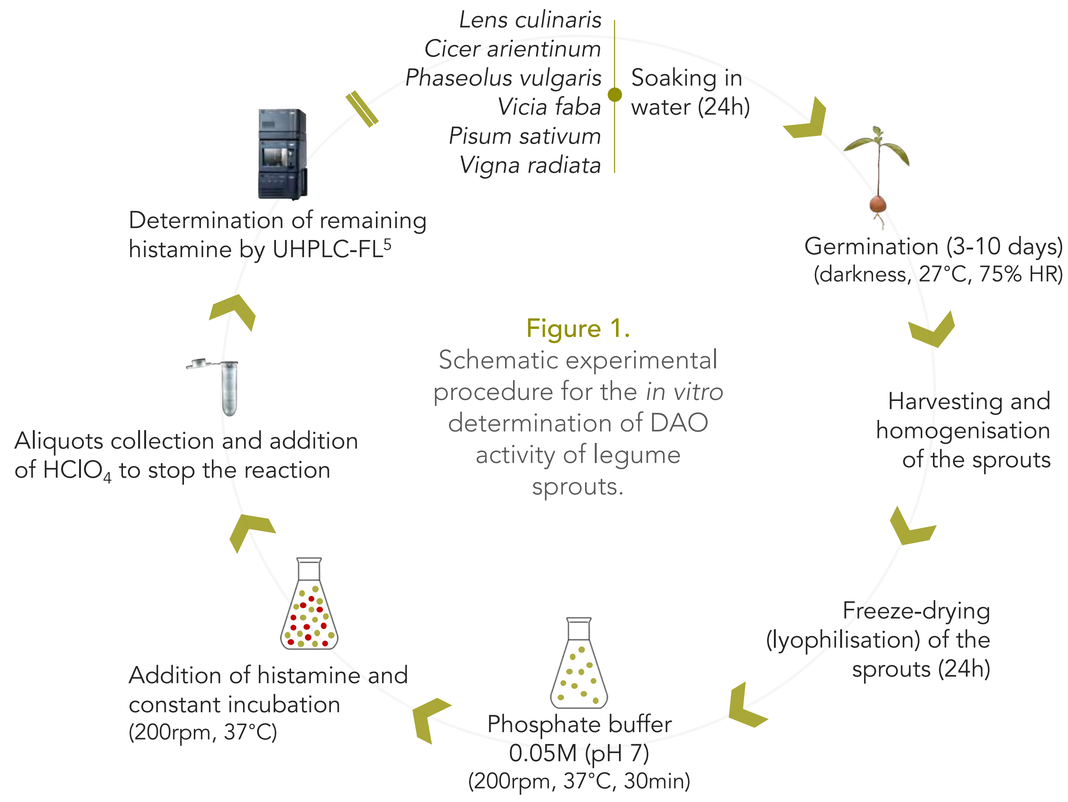

Figure 1. Schematic experimental procedure for the *in vitro* determination of DAO activity of legume sprouts.

RESULTS

Histamine-degrading capacity of legumes

Histamine-degrading capacity was found both in raw pulses and fresh sprouts of some legumes. DAO activity determined in raw pulses ranged from 0.32 to 1.95 mU/g, while this enzymatic activity reached values 20-fold higher in etiolated (grown in darkness) fresh chickpea sprouts. This enzymatic activity was absent in sprouts of mung beans, white, black, kidney and DOP *Ganxet* beans.

DAO activity of lyophilised legume sprouts

Lyophilisation of legume sprouts provided a concentrated, homogenised and manageable product. This low temperature dehydrated treatment did not affect the enzymatic activity of the sprouts (Figure 2).

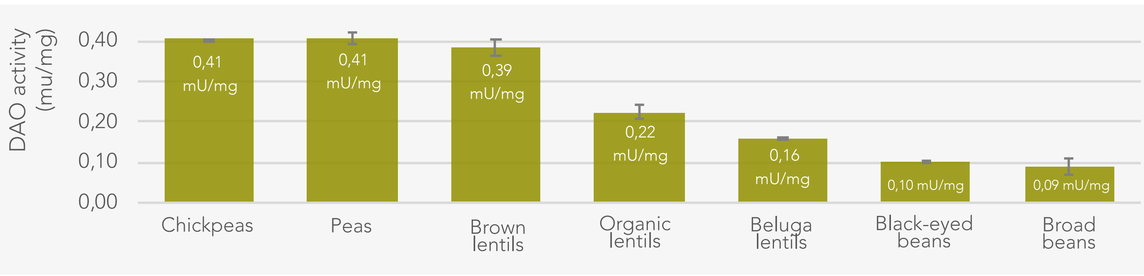

Figure 2. DAO activity of lyophilised legume sprouts grown in darkness.

Influence of sprouting duration on DAO activity of legume sprouts

DAO activity showed its higher levels at 3 days old etiolated sprouts. Longer sprouting period resulted in a decrease of this enzymatic activity (Figure 3).

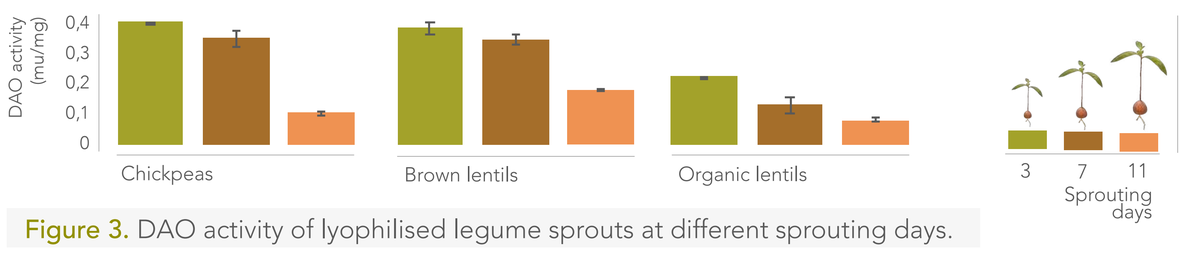

Figure 3. DAO activity of lyophilised legume sprouts at different sprouting days.

Influence of light exposure on DAO activity of lentil sprouts

Lyophilised 3 days old brown lentil sprouts showed maximum DAO activity when grown in darkness. The exposure to increasing light conditions supposed a decrease in the enzymatic activity (Figure 4).

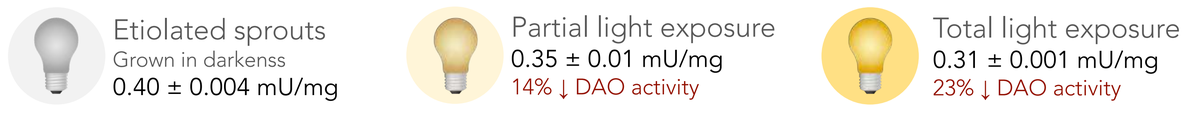

Figure 4. Influence of light exposure on DAO activity of 3 days old lyophilised lentil sprouts.

CONCLUSIONS

The ability of various raw pulses and legume sprouts to reduce histamine *in vitro* has been confirmed. Germination of the seedlings during 3 days in darkness has proven to ensure maximum DAO activity levels. The lyophilisation treatment allowed to concentrate and homogenise this vegetal tissue while preserving full histamine-degrading capacity. These results could indicate the potential of legumes as a plant source of the DAO enzyme.

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Conflict of interest:
Authors declare no conflict of interest.

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LOW-HISTAMINE DIET SUPPLEMENTED WITH EXOGENOUS DIAMINE OXIDASE ENZYME IS USEFUL FOR TREATING MIGRAINE IN PATIENTS WITH DAO DEFICIENCY

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INTRODUCTION

Low-histamine diets and/or exogenous diamine oxidase (DAO) supplementation are currently used to treat symptoms of histamine intolerance (HI), a disorder in histamine homeostasis that increases histamine plasma levels, mainly due to DAO deficiency. Headache is the most recognized symptom.

OBJECTIVE

To assess the effectiveness of a low-histamine diet combined with DAO enzyme supplement in migraine subjects with DAO deficiency.

METHODS

An intervention study was carried out in 212 individuals with a migraine diagnosis by a neurologist according to the International Classification of Headache Disorders and with DAO deficiency (DAO <80 HDU/ml). **Figure 1** schematize the study protocol. Subjects followed a 3-month low-histamine diet based on the exclusion of foods considered rich in histamine or other biogenic amines (**Table 1**) and usually related to the onset of HI symptoms. Moreover, a DAO supplement was administrated 20 minutes before breakfast, lunch and dinner. Outcomes assessed were duration and number of attacks and perception of pain intensity with a score-scale from 0 (absence) to 10.

Figure 1 Study protocol

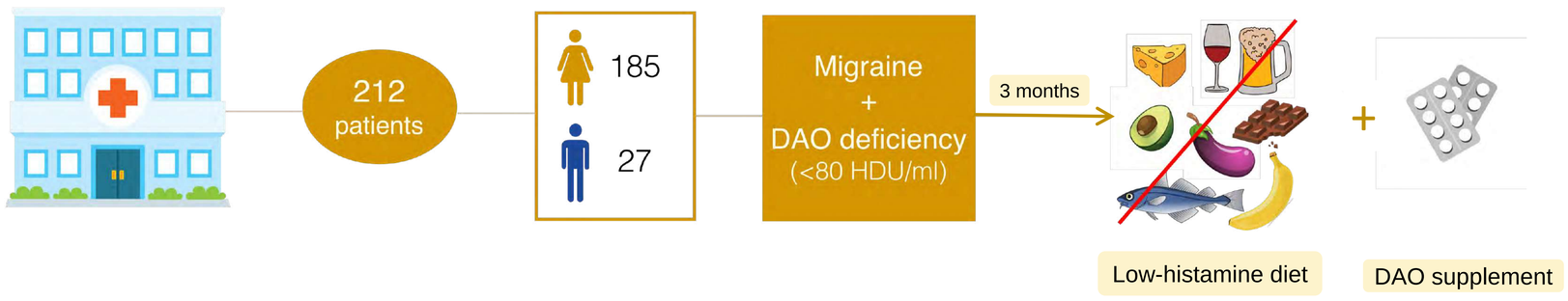


Table 1 Food to avoid in a low-histamine diet

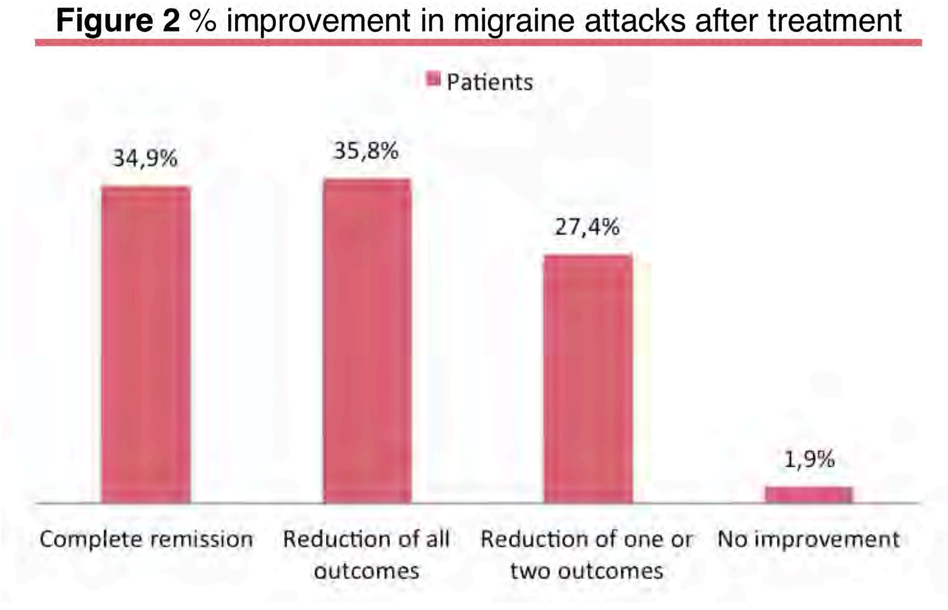
Alcoholic fermented beverages: beer, champagne, wine
Dairy products: milk, yogurt, cured cheese, cream
Dry-fermented sausages: ham, chorizo, *sobrasada*…
Eggs: egg white
Fats: Chocolate
Oily fish (fresh and canned) and shellfish: salmon, tuna, sardine, mussels…
Vegetables, legumes and fruits: spinach, tomato, eggplant, pumpkin, zucchini, chucrut, avocado, citrus fruits, bananas, kiwi, strawberries, pineapple, nuts and fermented soy derivatives

RESULTS

Most subjects showed an improvement in migraines after the 3-month treatment: 34.9% reported complete remission and another 35.8% had a reduced number of migraine episodes per month with less duration and pain intensity. The treatment was less successful in 27.4% of patients, because they just improve one or two of the outcomes assessed. Only 1,9% of patients did not improve at all (**Figure 2**).

On average, when comparing baseline and final values after treatment, all outcomes were significantly reduced: from 8 to 2 attacks per month, from 24 to 3 hours of pain and from 8 to 4 in pain intensity scoring.

Figure 2 % improvement in migraine attacks after treatment



CONCLUSION

A low-histamine diet supplemented with DAO enzyme for three months was useful in reducing the number of attacks, duration and intensity of pain in migraine patients with DAO deficiency. These preliminary results need to be confirmed in further double-blind, placebo-controlled, randomized clinical trials.

References: [1]Rosell-Camps et al. (2013); [2]Wagner et al. (2017).
Conflict of interest: Authors declare no conflict of interest.
Key words: DAO / histamine / low-histamine diet / migraine.

ACTIVIDAD DIAMINO OXIDASA (DAO) SÉRICA EN PACIENTES MIGRAÑOSOS

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INTRODUCCIÓN

La intolerancia a la histamina se debe a un desequilibrio en la homeostasis de la histamina, debido a una reducción de la actividad diamino oxidasa (DAO) intestinal, causando su acumulación en plasma[1]. Algunos autores han relacionado el déficit de DAO con enfermedades de alta prevalencia en la población, como migraña, enfermedades gastrointestinales (diarrea, enfermedades inflamatorias intestinales o dermatológicas (eccema, urticaria, dermatitis atópica)[1,2,3].

OBJETIVO

El objetivo de este trabajo fue determinar la prevalencia del déficit de DAO en pacientes con diagnóstico confirmado de migraña según los criterios actuales de la *International Headache Society* (IHS) y en voluntarios no migrañosos.

METODOLOGÍA

El estudio se realizó en la Unidad de Cefalea del Hospital General de Cataluña con un total de 198 voluntarios, divididos en dos grupos: un grupo de 137 pacientes diagnosticados de migraña por la Unidad de Cefalea del Hospital de acuerdo con los criterios actuales de la IHS, y un grupo control integrado por 61 voluntarios sin diagnostico clínico de migraña (Figura 1).

Las muestras de sangre se recogieron mediante punción venosa en un tubo con EDTA después de un período de ayuno de 8 horas. La actividad DAO se determinó mediante un test ELISA, considerando que valores inferiores a 80 HDU/ml (Unidades Degradadoras de Histamina/ml) eran indicadores de déficit de DAO (Figura 1).

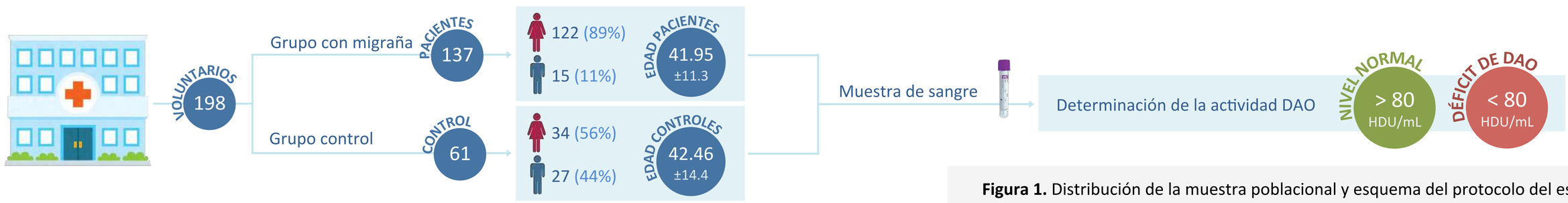

Figura 1. Distribución de la muestra poblacional y esquema del protocolo del estudio.

RESULTADOS Y DISCUSIÓN

La prevalencia del déficit de DAO fue muy elevada en el grupo de pacientes con migraña, con un 87% de personas con este déficit enzimático (Figura 2). El valor medio de actividad DAO en pacientes con migraña fue del 64.5 ±33.5 HDU/mL, valor significativamente inferior ($p<0.0001$) al obtenido en voluntarios sanos (91.9 ± 44.3 HDU/mL) (Figura 3). El diagrama de cajas (Figura 4) muestra de forma gráfica la distribución de los valores de actividad DAO en ambos grupos, observándose una menor variabilidad en el grupo con migraña. La amplia variabilidad en el grupo control sugiere que quizás fuera conveniente estudiar con más precisión los valores normales de actividad DAO.

El 13% de la población migrañosa sin déficit de DAO y el 44% de la población no migrañosa con déficit de DAO indican que esta deficiencia enzimática puede ser un factor que predispone a la migraña, pero no la única causa. Como es sabido, la migraña tiene una etiología multifactorial. La migraña es uno de los síntomas frecuentemente asociados a la intolerancia a la histamina, pero no el único[1,2,3]. En este estudio no se registró la presencia de otros síntomas asociados a esta intolerancia y, por tanto, no se puede concluir que el 44% de pacientes que muestran déficit de DAO en la población no migrañosa sean realmente asintomáticos para la intolerancia a la histamina.

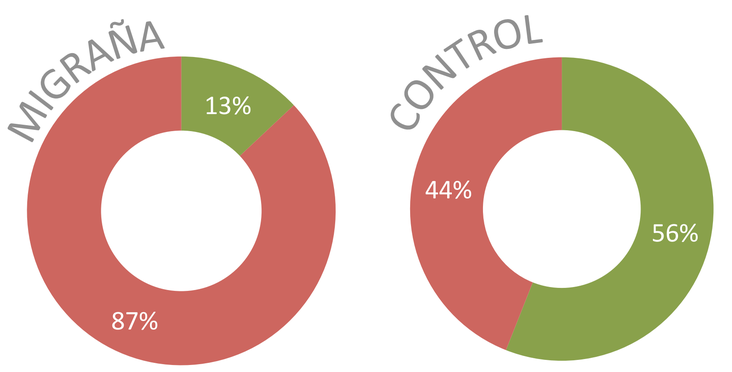

Figura 2. Porcentaje de déficit de DAO en pacientes migrañosos (izquierda) y en grupo control (derecha).

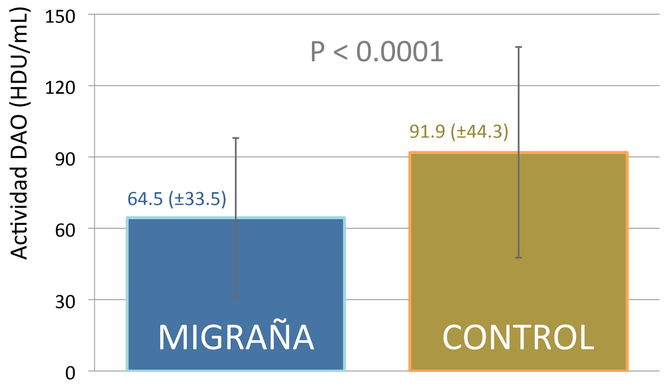

Figura 3. Actividad DAO en pacientes con migraña (n=137) y población control (n=61).

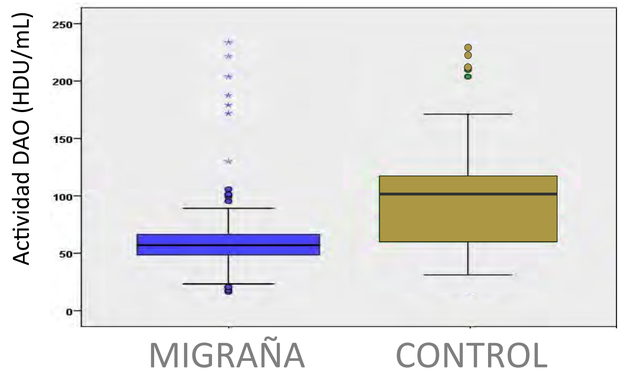

Figura 4. Diagrama de cajas de la distribución de los valores de actividad DAO en pacientes migrañosos y controles.

CONCLUSIONES

El déficit de actividad DAO fue más frecuente en pacientes con migraña que en la población control. Un 13% de los individuos correctamente diagnosticados de migraña presentaban niveles normales de actividad DAO. Son necesarios más estudios con el fin de conocer con más precisión los valores normales de actividad DAO en población sana para, entre otras cosas, poder establecer con más evidencia la correlación entre el déficit de DAO y la aparición de migraña.

Bibliografía: 1: Maintz y col., Am J Clin Nutr 2007; 85:1185. 2: Schwelberger. Inflamm. Res 2010; 59:219. 3:Kovacova-Hahanuskova y col. Allergol Immunopathol 2015; 43:498.

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DIETARY TREATMENT AND BLOOD DIAMINOOXIDASE (DAO) VALUES IN CYCLIC VOMITING SYNDROME (CVS)

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INTRODUCTION

Certain authors consider CVS as a variant of the headache/migraine, due to a disorder of the brain gut axis. In DAO enzyme deficiency, histamine is not removed and accumulates, causing various disorders, being the most common and disabling migraine headaches. The consumption of certain histamine rich foods (proteins and fats) may be associated with these symptoms.

AIMS

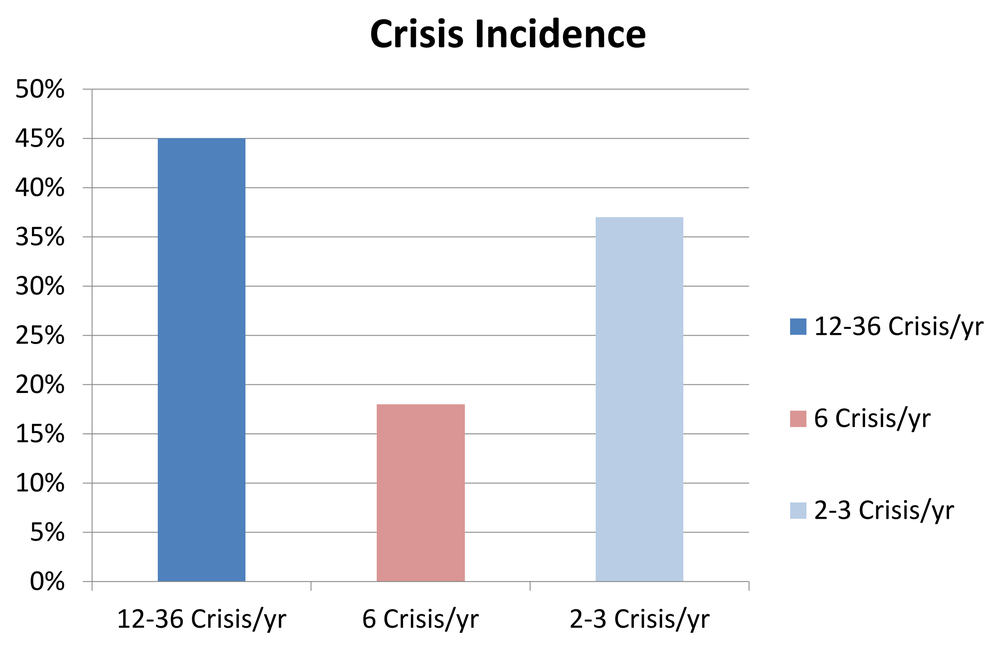
Determine values in blood of the DAO enzyme in CVS patients. Assess its evolution after prescription of a low fat and protein diet.

MATERIAL AND METHODS

Interventional prospective descriptive study in CVS patients. We determined the DAO value and dietary interview was realyzed. All of them recieved fractional and low fat and protein diet (fat <30% TC and protein <12%TC) and dietary counseiling, as well as specific pharmacological treatment where necessary. Patients with low and intermediate DAO values, were supplemented with (DAOSIN®/MIGRASIN®: 1-3 capsules/day). Low DAO values<40 HDU/ml; Intermediate DAO values 40-80 HDU/ml; normal DAO values >80 HDU/ml.

RESULTS

- 11 patients presented vomiting and migraines .
- 8 of them, were diagnosed CVS.
- Mean of age: 28,3±6; 6 women.
- DAO values:
  - we found intermediate/low activity in 100% of vomiting and migranies patients.
  - In 3 patients not diagnosed of CVS presented intermediate activity values.



- After the nutritional support with low fat, a fractional diet and DAO supplementation, we found a drastic reduction of the crisis:
  reduction of 100% in patients with vomiting and migraines, and crisis reduction of 70% CVS patients.
  We observed correct adherence to dietary advice provided in all patients.

CONCLUSIONS

In CVS patients low fat and protein diet must be recommended. DAO supplementation may improve their follow-up.

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DIETARY TREATMENT AND BLOOD DIAMINOOXIDASE (DAO) VALUES IN PATIENTS AFECTED VOMITING AND OTHER GASTROINTESTINAL AND NEUROLOGICAL SYMPTOMS

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Gastroenterology and Nutrition Unit Via Augusta. Barcelona. Spain.

INTRODUCTION

Migraine is a complex disease, which is suspected of a major genetic component in its origin. Headache is the most common clinical sign and is usually accompanied by nausea and vomiting. It is estimated that it affects between 12 and 17% of the population. In DAO enzyme deficiency, histamine is not removed and accumulates, causing various disorders, being the most common and disabling migraine headaches. The consumption of certain histamine rich foods (proteins and fats) may be associated with these symptoms.

AIMS

To determine values in blood of the DAO enzyme in patients with frequent episodes of migraines, vomiting and abdominal pain. Assess its evolution after prescription of a low fat diet.

MATERIAL AND METHODS

Interventional prospective descriptive study in patients presenting gastrointestinal symptoms.
We distinguishing two groups:

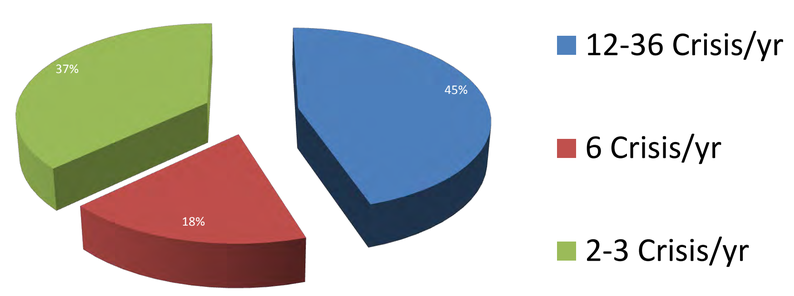
- First group presenting vomiting and migraines
- Second group presenting abdominal pains and migraines.

In all of them DAO levels and a dietètic review were determined. All of them recieved fractional and low fat diet (<30% TCA) and dietary counseiling, as well as specific pharmacological treatment if necessary.
Patients with low and intermediate DAO values, were supplemented with (DAOSIN®/MIGRASIN®: 1-3 capsules/day). Were consideered: low DAO vàlues:<40 HDU/ml, Intermediate DAO values 40-80 HDU/ml, normal DAO values >80 HDU/ml.

RESULTS

- 32 patients were included, all of them with gastrointestinal symptoms.
- Mean of age: 26,3±18,8;22 women.
- Symptoms:
  - 11 patients presented vomiting and migraines
  - 21 patients presented abdominal pains and migraines (not vomiting).
- In patients who experienced abdominal pains and migraines, we found a direct relationship between migraine headache and abdominal pains, with an incidence of 2 crisis/month.
- After pharmacological treatment and nutritional support with low fat and fractional diet, we found a drastic reduction of the crisis: with a reduction of 80% in patients with vomiting and migraines and a reduction of 50% in patients with abdominal pains and migraines.

Crisis incidence in patients presenting vomiting and migraines



	DAO Activity	
Patients	Intermediate-Low values	Normal values
Vomiting and Migraines	100%	0%
Abdominal pain and Migraines	86%	14%
Total of patients	91%	9%

CONCLUSIONS

Chronic migraine patients with gastroenterology symptoms, tend to have DAO enzyme below normal figures. Most of ours patients presented low DAO values. With specific drug treatment and a proper dietary advice (low fat diet) these patients can improve their symptoms and contribute to improve their quality of life.

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XVI REUNIÓ DE LA SOCIETAT CATALANA DE NEUROLOGIA

XXVI CURS D'ACTUALITZACIÓ EN NEUROLOGIA

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La Fundació de la Societat Catalana de Neurologia atorga l'accèssit a la segona millor Comunicació-Pòster presentada durant la XVI Reunió Anual de la Societat Catalana de Neurologia i el XXVI Curs d'actualització en Neurologia a:

En/Na

Izquierdo, J. , Soler, Ll. , Balaguer, E. , Mon, D.

Amb la Comunicació:

DÈFICIT DE DIAMINOOXIDASA (DAO) COM A PREDISPOSANT DE LA MIGRANYA

Dr. Adrià Arboix
President de la Societat Catalana de Neurologia

Vic, 15-16 de març de 2012

Evaluation of DiAmine Oxidase deficiency in patients with migraine

Vidal Carou • A1, Sabater Sales • B2, Titus Albareda • C3

(A1) Nutrition and Bromatology Department of University of Barcelona. **(B2)** MD in Pharmacy. Responsible for scientific research at Sabater Analysis (LABCO). **(C3)** Honorary Member of the Spanish Society of Neurology, and Scientific Advisor of the Spanish Association of Headache Patients, AEPAC

Introduction

Migraine is a complex, recurrent and disabling disease, but its etiopathogeny remains unknown, so it is necessary to find new approaches to the biochemical mediators of the disease. Histamine, one of the most important mediators, is mainly metabolized by **DAO (Diamine Oxidase enzyme)**. Therefore, it is believed that a decrease in DAO activity may cause histamine excess, increasing the risk of suffering from clinical pictures such as migraine, among others.

Objective

To determine the rate of migraine patients presenting DAO deficiency compared to the observed rate in general population without migraine.

Method

Target population consisted of migraine volunteers. Healthy controls were selected among volunteers of their micro social environment. Given a total sample size of 160 individuals, the studied aimed to determine **DAO** activity level in the participants by analytical determination using a validated procedure.

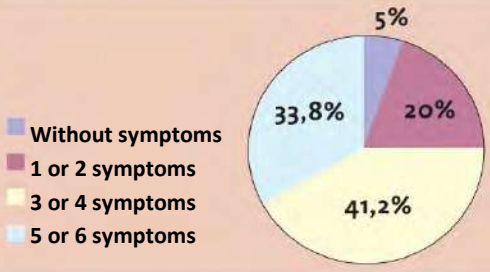
Materials

IHS diagnostic sheet, patient information sheet, informed consent form, 160 analytical tests to determine **DAO**, data collection logbook, documents for the registration in the data protection agency.

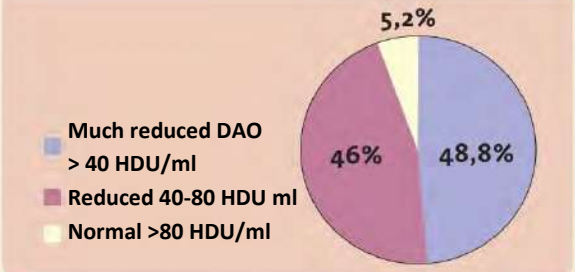
Results

48.8 % of migraine patients presented values of much reduced **DAO** activity, and 46% of reduced activity. 95% of migraine patients experienced other symptoms related to histamine intolerance. The average value obtained for **DAO** activity is lower in the migraine group, with a significant difference (p=0.001).

MIGRAINE PATIENTS WITH OTHER HISTAMINE SYMPSTOMS



VALUE OF DAO ACTIVITY IN MIGRAINE PATIENTS



Conclusion

The results confirm the scope of the research and show that **DAO activity is significantly lower in migraine patients compared to controls.**

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