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Urinary histidine excretion in patients with classical allergy (type A allergy), food intolerance (type B allergy), and fungal-type dysbiosis

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Changes in histidine excretion reflect histidine conservation and thus the level of histamine secretion. Low levels were found in untreated patients with atopic (type A) allergy. However, levels in food intolerance (type B allergy) and fungal-type dysbiosis were also low ($P < 0.001$ for each group compared with nonallergic controls). There were no differences between the three groups. The biochemical and clinical significance of these findings is discussed. (J. Nutr. Biochem. 9:586–590, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Over 60 years ago it was found that children with allergic asthma had hypochlorhydria.[1] On allergen exposure, classical allergic diseases are accompanied by the release of histamine from granules in sensitized mast cells or gut basophils. Histamine is formed in the body from the amino acid histidine: After its release in an allergic reaction it is metabolized to 1:4 methyl histamine and thence to 1 methyl-4-imidazole acetic acid, or to imidazole acetic acid or N-acetyl histamine. These are excreted in urine and lost to the circulation. Histidine is a major component of muscle protein. In natural turnover it is catabolized to 3-methyl histidine, which is excreted in urine.[2] Blood histidine levels appear to be almost constant.[2] In active allergy histamine requirements are elevated and this need must be fulfilled by changes in histidine metabolism. As yet this issue seems to have received little study.

The present study is based on an anecdotal observation of lowered urinary histidine excretion in untreated allergic patients.[3] In patients with classical allergic conditions, measurement of this phenomenon may be of less use to clinicians because there is a wealth of available laboratory indices, based on immunoglobulin E (IgE) levels[4] and direct skin and challenge tests. These tests are valid in both inhalant and food sensitivity if IgE is involved, causing reactions that are rapid in onset and of short duration. Currently, these conditions are given a variety of names, either organ specific, such as allergic rhinitis, or generic, such as food allergy.[5] Recently, a new nomenclature has been proposed that combines all these responses under one heading as *type A allergy*.[6] This system will be used in this report. The term *food intolerance* is often used for all non-IgE mediated types of adverse reactions to food, including those due to inborn metabolic errors,[7] psychological factors (food aversion),[8] and pharmacologic mediators in foods.[9] Recently, some authors have modified this definition to exclude an immunologic cause.[10] However, there is a large group of adverse reactions that have a common natural history, and other authors consider that the presumptive evidence that allergic mechanisms are concerned warrants the term *type B allergy*.[6] This general term covers all the nonatopic forms of allergic response and will be used here.

Our study also is concerned with adverse reactions to certain foods that seem to be related to disturbances of the gut microflora (fungal-type dysbiosis). Although there are earlier references to this condition, the first systematic description is that of Hurst and Knott[11] in 1930, who termed it *intestinal carbohydrate dyspepsia*. In the 1950s this term

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fell into disfavor because of lack of research. In 1980, it was revived by Truss, although from his papers it is clear that he was unaware of the earlier descriptions:[12] He found that antifungal drugs helped and attributed the condition to *Candida albicans*. This suggestion, never confirmed by any published research, may well have been the chief factor mitigating against general acceptance of the condition, and it is in an effort to avoid the prejudgments the name suggests that the term *fungal-type dysbiosis* has been coined.[13] The symptom complex includes any or all of irritable bowel, cattarh, and neuropsychiatric (minimal brain dysfunction) symptoms, to which may sometimes be added clearly allergic responses such as urticaria.[14] The condition is justified by the beneficial therapeutic response to a regime consisting of a diet low in fermentable, yeasty, and mold-containing foods, with or without antifungal drugs.[14] Since 1990 studies have identified some characteristics of the condition that provide further justification for its physical existence. When submitted to a fasting sugar challenge, subjects with the condition produce excess ethanol,[15,16] which usually normalizes after treatment.[14] A few patients do not show excess ethanol levels, but have increased urinary B alanine excretion.[17] In untreated patients B vitamins, zinc. and magnesium are low.[18] Sugars are handled differently from those with type B allergy[19] and gut permeability is increased.[20]

We considered that it might be interesting to compare histidine excretion in these three conditions.

Materials and methods

Patients

Sequential patients newly referred to one of the authors (KKE) were admitted to the survey program. Those with a type A allergy were invited to contribute samples, as were those whose diagnoses were considered to be either type B allergy or fungal-type dysbiosis. It can be difficult at a clinical level to distinguish these two entities at first presentation and patients were entered in the appropriate category according to the results of investigation. A majority tested positive for ethanolic fermentation and therefore were allotted to the fungal-type dysbiosis group; only a minority, who tested negative on this test but had positive reactions to elimination and challenge dieting, were classed as having type B food allergy. Consequently, entry to the fungal study arm was closed after 21 patients had been entered, and we continued to recruit to the other arms.

The results of a survey such as this could be affected by an abnormal dietary intake of the nutrient concerned. Protein consumption (and hence histidine intakes) were at similar levels for all three patient groups and for the control subjects.

The opportunity was also taken to examine specimens from a modest number ($N = 8$) of patients with fungal-type dysbiosis who at the time of testing had already been undergoing treatment for their condition and were clinically responding.

Controls

A key part of this study was to produce a more precise normal range. The previous normal range for histidine excretion did not account for allergic status. In this study, in addition to the usual criteria that the subjects should be healthy, on a normal diet, and not receiving medication or nutritional supplementation we added these further restrictions:

1. No malaise presently or recently for at least one month. A rigorous clinical historical survey to exclude allergic conditions.
2. These subjects were drawn from another project[21] and because of this it was not possible to measure IgE status in this group.

Laboratory assays

Total IgE levels were performed by the IMx microparticle enzyme immunoassay technology (Abbott Laboratories, Diagnostics Division, Abbott Park, IL USA).[22] Ethanol fermentation was performed by a gas chromatography technique as reported previously.[17,18,20]

Although the methodology for the measurement of urinary histidine has been published,[3] because it is central to this current work it is reproduced here. Amino acid analyses were performed on aliquots of 24 hour urine samples using a fully automated gradient elution high performance liquid chromatography (HPLC) system with flourometric detection (Gilson Medical Electronics, Villeres-le-Bel, France). The separation was carried out on a C18 microsorb reverse-phase column (Rainin Instrument Co. Inc., Woburn, MA, USA) following pre-column derivization with the flourogenic reagent o-pthaldehyde-2-mercaptoethanol (OPA-MCE), as in the method of Turnell and Cooper.[23] The mean run inter-batch coefficient of variation for histidine was 6%.

Statistical methods

Histidine levels were summarized by the median for each group and compared between the five groups using the Kruskal-Wallis test. Because this overall test was statistically significant, individual groups were compared with the controls using the Mann-Whitney test. The statistical analyses of these data were performed using the software packages Microsoft Excel (Microsoft UK Ltd, Wokingham, Berks, UK) and SPSS (SPSS UK Ltd, Woking, Surrey, UK).

Results

Normal values

Normal values were derived from a group of 25 normal nonallergic volunteers, 11 males and 14 females, whose ages ranged from 26 to 45 years, with a median age of 32 years.[21]

The previously used[3] normal range for urinary histidine excretion has been 280 to 2,100 μmol/24 hours, comparable with that for other laboratories. In our more rigorously defined control group, the mean excretion was 1,072 μmol/24 hours, standard deviation 360. Thus, we are able to use a standard protocol of two standard deviations from the mean to produce a new normal range of 371 to 1,771 μmol/24 hours. The individual results are shown in *Table 1*.

Study groups

The results for new patients are shown in *Table 2*. There were 20 patients with food intolerance (type B allergy), 14 patients with classical allergy (type A allergy), and 21 patients with fungal-type dysbiosis. The individual results for urinary histidine excretion and total IgE are listed. When the four groups are compared with the control group (initially using the Kruskal-Wallis test, and then because this was statistically significant, the Mann-Whitney test) the excretion of histidine was significantly lower in all three

Table 1 Normal values for 24 hour urinary histidine excretion

Patient number	Histidine (μmol/24 hr)
1	1,015
2	1,254
3	1,156
4	980
5	852
6	1,015
7	1,254
8	1,953
9	882
10	1,015
11	1,457
12	1,500
13	980
14	352
15	465
16	889
17	900
18	1,098
19	1,078
20	1,688
21	1,500
22	785
23	655
24	900
25	1,200
Median	**1,015**

new patient groups ($P < 0.001$) than the control group, but clearly not from each other. The treated patient group (shown in *Table 3*) with a median of 956 μmol/24 hours is no different from the control group.

Discussion

Earlier researchers have generally considered a range of amino acids and consequently histidine has not received detailed individual attention. A standard protocol for establishing reference levels uses volunteers who fulfill certain criteria: They must be healthy, on a normal diet, and not receiving medication or nutritional supplementation. For other amino acids these parameters may suffice; however, for histidine with its role in allergic reactivity, atopic status is also important, especially in view of the frequency of allergic diseases in western populations. In 1979, one of the authors (KKE) found an overall incidence of 29.79%,[24] higher for all allergic conditions than when the same population had been previously assessed in 1974 (22.85%). Subsequent surveys have shown even higher figures.[25] In many cases both patients and their medical advisors are unaware that they have an allergic illness; a common but often ignored symptom is nonspecific malaise.[26] Thus, to derive an allergy-free "normal" reference range we needed to adopt stricter criteria.

The normal range derived in this way was narrower. We must consider what factors cause histidine levels to deviate from the mean.

These data show that normal values of urinary histidine excretion should be based on findings in normal symptom-free nonallergic individuals. In our data this gave a normal range of 371 to 1,771 μmol/24 hours, but we recognize that this may be artificially extended by the limited number of fit healthy nonallergic controls to which we had access. We hope that others will confirm the dependence of histidine excretion on allergic status in a larger series.

Histidine is an essential amino acid in childhood; in adults it is classed as a protein amino acid, but there are doubts about the extent to which it can be synthesized. Allergy, when active, involves the release of histamine from mast cells and gut basophils; as seen above, this causes a loss of histamine to the system. This must be replaced by a continuing synthetic process from histidine. Because muscle histidine is not available for this process and blood histidine levels are homeostatically controlled, this must be effected by conservation including increased renal tubular reabsorption of histidine from dietary sources Because dietary uptake is unlikely to increase. Histidine above body requirements is excreted in the urine. In deficit this loss can be recouped by an increase in proximal tubular renal reabsorption. In severe active allergic disease this alone may not be sufficient to meet the needs of the system. The seminal study by Bray,[1] conducted and originally published in 1931, drew attention to the existence of hypochlorhydria in children with asthma and in some of their allergic parents. At the time the biochemical significance of these findings was not apparent because histamine had not yet been identified. When it was, this study was no longer at the forefront of researchers' minds and no connection was made. Peptic hydrochloric acid secretion depends on gastric histamine production. In active allergy it seems likely that peptic histamine secretion is reduced as a second stage mechanism for histidine conservation, when urinary recovery of histidine in patients with active allergy is insufficient to meet metabolic needs. The resulting hypochlorhydria would depress gastric protein catabolism and diminish the availability of essential amino acids from diet. This would have the unwanted effect of exacerbating the diminished histidine status.[27] In routine clinical management we do not see hypochlorhydria in the milder cases, indicating that this is likely to be an effect caused by deficiency.

In the course of this work we have had the opportunity to measure urinary histidine excretion in some other patients who have been receiving treatment for their allergies and for fungal-type dysbiosis. The levels in treated gut dysbiosis patients are clearly higher than in the untreated group and not distinguishable from normal values. However, in a few patients with allergies (not part of this series) we have noted that the reduced rate of urinary loss before treatment tends to overswing to an enhanced level before settling at about the mean, but the timing of these events appears to vary between patients. We hope to follow this up with sequential observations.

Our results for type A allergies, type B allergies, and fungal-type dysbiosis are all below the normal mean and the difference is very highly statistically significant. All three groups gave mean values only just above the lower limit of the normal range for nonallergic individuals with no differences between them.

As expected, "classical" (type A) allergy patients showed lowered levels of urinary histidine loss. That patients with type B allergies and fungal-type dysbiosis also showed

Table 2 Urinary histidine[1] excretion and immunoglobulin G (IgE) levels in new untreated patients with food intolerance, atopic allergy, and fungal-type dysbiosis

Histidine/IgE: Food intolerance			Histidine/IgE: Atopic allergy			Histidine/IgE: Fungal-type dysbiosis		
Patient	Histidine	IgE	Patient	Histidine	IgE	Patient	Histidine	IgE
1	270	47	1	310	41	1	300	46
2	280	476	2	350	74	2	295	1,456
3	295	482	3	350	33	3	973	23
4	1,060	84	4	375	958	4	376	53
5	580	22	5	470	143	5	217	53
6	372	21	6	870	68.4	6	536	3.6
7	200	56	7[2]	496	758	7	396	19.4
8	867	20	8[2]	430	41	8	595	2,044
9	325	144	9	190	17	9	280	5
10	300	24	10	1,105	89.6	10	205	394
11	220	64	11	220	173	11	320	50
12	395	89	12[2]	430	41	12	637	13
13	180	986	13	350	179	13	259	5.8
14	520	30	14[2]	500	82	14	760	37.2
15	963	56				15	300	7
16	220	64				16	825	92
17	693	155				17	625	34
18	565	11				18	256	180
19	800	40				19	370	69
20	827	2,262				20	895	8
						21	720	29
Median	383.5	60	Median	402.50	78.00	Median	376.00	37.20
Min	180	11	Min	190	17	Min	205	3.6
Max	1,060	2,262	Max	1,105	958	Max	973	2,044
N	20		N	14		N	21	
r	0.0914		*r*	−0.0504		*r*	−0.0685	

Comparison of histidine values in the three groups with control group (Mann-Whitney U test)[3]

	P-value
Food intolerance vs. controls	<0.001
Atopic allergy vs. controls	<0.001
Fungal-type dysbiosis vs. controls	<0.001

[1] All histidine results expressed in μmol/24 hr.
[2] Child.
[3] In a control patient study the median urine histidine was 1,015 μmol/24 hr (n = 25) (see Table 1).

Table 3 Urinary histidine[1] excretion and immunoglobulin G (IgE) levels in fungal-type dysbiosis—results on treated patients (recovering)

Patient	Histidine	IgE
1	927	9.4
2	1,100	31
3	985	25
4	795	6
5	1,230	18
6	420	5
7	450	121
8	1,220	10
Median	956.00	14
Min	420	5
Max	1,230	121
n	8	
r	−0.4401	

Comparison of median histidine level with patients from Table 2

Food intolerance	*P* = 0.005
Atopic allergy	*P* = 0.006
Fungal-type dysbiosis	*P* = 0.002

[1] All histidine results expressed in μmol/24 hr.

values that were just as low is a new observation. It is known[28] that patients with type B allergies do release histamine locally in the gut, but no data are available for this. That the findings are similar to diseases with a proven allergic mechanism does not in itself establish that these two conditions involve allergic mechanisms. Other pathways involving histamine metabolism that do not involve allergy could be involved. However, in medicine commonest things are always commonest and because of the high incidence of allergy in the western world the possibility that both type B allergy and fungal-type dysbiosis involve allergic processes remains the most likely explanation until proved otherwise.

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