

ORIGINAL PAPER

Incremental build-up food challenge – a new diagnostic approach to evaluate pseudoallergic reactions in chronic urticaria: a pilot study

Stepwise food challenge in chronic urticaria

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Summary

Background The remission rate of patients with chronic urticaria (CU) due to elimination diets varies between 31% and 71%. However, the diagnostic value of subsequent traditional oral provocation tests with food additives in capsules remains unsatisfactory.

Objectives A newly incremental build-up food challenge (IBUF) for patients with CU was designed and implemented in an open pilot study. Primary endpoint was the percentage of patients developing urticaria during at least one step of IBUF after an initial complete remission due to a pseudoallergen-free elimination diet.

Methods In total, 153 patients with CU were submitted for 5 weeks to a pseudoallergen-free diet. All patients with remission were included to the 6-week IBUF protocol, containing pseudoallergen-rich foods in a systematic and additive manner. The recurrence and severity of CU was evaluated by urticaria score. Subjective disturbance and quality of life were evaluated by patients' diary, visual analogue scale and quality of life questionnaire (CU-Q₂oL). Subsequently, patients were followed up for 3–24 months after IBUF by a telephone interview. **Results** A total of 104 patients completed the pseudoallergen-free diet, whereby 51% reported partial, 17% complete and 32% no remission due to the diet. All diet responders showed a decrease in subjective impairment, urticaria and quality of life score ($P < 0.001$ each). Eighty-six percent (12/14) of the patients reaching complete remission, showed a recurrence of urticaria symptoms during the IBUF protocol. Fifty-eight percent (7/12) of these patients still remained free of symptoms due to avoidance of IBUF-identified foods at telephone follow-up. In patients with partial remission to pseudoallergen-free diet, however, IBUF did not provide information about the cause of urticaria symptoms.

Conclusions The newly developed IBUF protocol seemed to be a promising method for identifying individually incompatible foods in some CU patients. IBUF should be verified by randomized controlled trials to gain additional evidence for its diagnostic value.

Keywords additives, chronic urticaria, diet, elimination diet, food challenge, IBUF, oral provocation diet, outpatient, pseudoallergens, pseudoallergic

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Introduction

Chronic urticaria (CU) is affecting 0.1–3% of the population in the USA and Europe. The classification is based on duration, frequency and causes. CU can be related to infectious or inflammatory processes, but is diagnosed as idiopathic in approximately 75% of the cases [1–3]. Hereunto include urticaria due to pseudoallergic reactions to food ingredients.

Remission rates due to elimination diets are reported in the range between 31% and 100%, comprising 'full' and 'partial' responders [4–17]. Only few publications exist with a clear differentiation between full and partial response. In many studies, diet responders are determined by a reduction in symptoms and/or medication intake and are not distinguished from full responders (non-existent of symptoms and no medication intake). A 'full' response after different dietary elimination interventions is

reported to be in the range between 31% and 71% [4, 5, 8, 9, 17].

To identify the eliciting agents, double-blind, placebo-controlled food challenge (DBPCFC) with food additives in capsules is the current gold-standard. Nevertheless, the outcome is unsatisfactory: only 14–19% of the diet responders show recurrence of urticaria due to this kind of challenge [11, 17, 18] and only 25% of patients, initially reacting to a mixture of pseudoallergens, develop symptoms when the same food additives are given separately [17]. A challenge with a combination of several pseudoallergens induces urticaria more frequently than a challenge with single pseudoallergens. Concrete: only in 3–4% of CU patients with a remission after an elimination diet, a causative food additive can be found. Hence, a clear recommendation for food avoidance is difficult if patients are symptom free among an elimination diet, but do not flare due to a capsule challenge.

Pseudoallergens evoking or enhancing urticaria are artificial food dyes, preservatives and sweeteners [11, 12, 19, 20], aromatic volatile compounds in tomatoes, herbs and wine [21] or other phenolic substances such as salicylic acid, *p*-hydroxy benzoic acid, anethole, ethyl vanillin, citron oil and orange oil [11, 12, 22, 23]. Alcohol and high dietary fat intake have been also described to elicit urticaria [10, 24–26]. The use of newly developed flavourings and food additives by the food industry is common, changes rapidly, and is not adequately reflected in the standardized capsule tests. In addition, consumption of supplemented convenience food is increasing [27].

Hence, we developed an incremental build-up food challenge (IBUF) with respect to realistic amounts of known and hitherto unknown pseudoallergens, contained in an unrestricted diet with respect to individual preferences and dislikes. Our pilot study was designed to investigate the effectiveness of this newly designed outpatient IBUF in patients with CU.

Materials and methods

Patients and diagnostic criteria

A total of 153 outpatients and inpatients (101 females and 52 males, age range 10–76 years, mean 42 years) were recruited between January 2005 and January 2007. Inclusion criteria were as follows: age between 7 and 80 years, urticaria duration of at least 6 weeks with frequency of symptoms at least once a week within the last 4 weeks. Exclusion criteria were isolated physical urticaria, cachexia, diabetes mellitus, pregnancy and nursing, because of a latent risk for malnutrition. All patients with a known hypersensitivity to foods which were essential in the diet were also excluded (e.g. lactose intolerance, sorbitol malabsorption or IgE-mediated food allergies). On admission, prick tests with common aeroallergens (tree and

grass pollen, house dust mites, cats and dander extracts) and food extracts (according to medical history) were performed in patients who did not take antihistamines. Cold, heat and pressure urticaria were excluded by patients' history and appropriate tests, if necessary [3].

Study design

This study was designed as an open-label pilot study with two different diet periods. In the first diet period, a pseudoallergen-free elimination diet (PAD) was followed over a period of 5 weeks to identify diet responders. Patients without any response were excluded. All diet responders went on to the second diet period with duration of 6 weeks to evaluate the development of urticaria during the IBUF (Fig. 1). Oral antihistamines and steroids were permitted during the entire study, but patients were advised to discontinue their medications during the first diet period (PAD) if symptoms resolved. All patients received a diary to document symptoms and medication intake. Subsequent to IBUF, patients were followed up by a single telephone interview within a time frame between 3 and 24 months after IBUF to monitor the long-term effect of IBUF.

The study was approved by the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität Münster), and written informed consent was obtained from each patient or the legal guardian.

Pseudoallergen free diet: first diet period

The PAD used in our study is based on the protocol described by Zuberbier *et al.* [11]. In our protocol, chives, onions and cold-pressed oils were additionally prohibited to avoid pseudoallergic reactions due to aromatic volatile compounds (Table 1) [21]. Patients were instructed to follow the diet over a period of 5 weeks, although a diet response has already been shown after 2 weeks, if no anti-allergic treatment was allowed [11]. This extended duration of PAD was chosen, because some patients rely on antihistamines and/or oral corticosteroids and were advised to reduce or discontinue this medication individually during the PAD. All patients were educated in detail by a dietician and received adequate cooking recipes.

Incremental build-up food challenge: second diet period

The IBUF protocol presented a new method to reintroduce foods in patients with a remission due to an elimination diet such as PAD. IBUF is basing on the fact that pseudoallergens act in a dose-dependent and cumulative manner. IBUF contained six 1-week steps with different cumulative food ingredients (Table 2). The sequence of the reintroduction of food is based on known eliciting

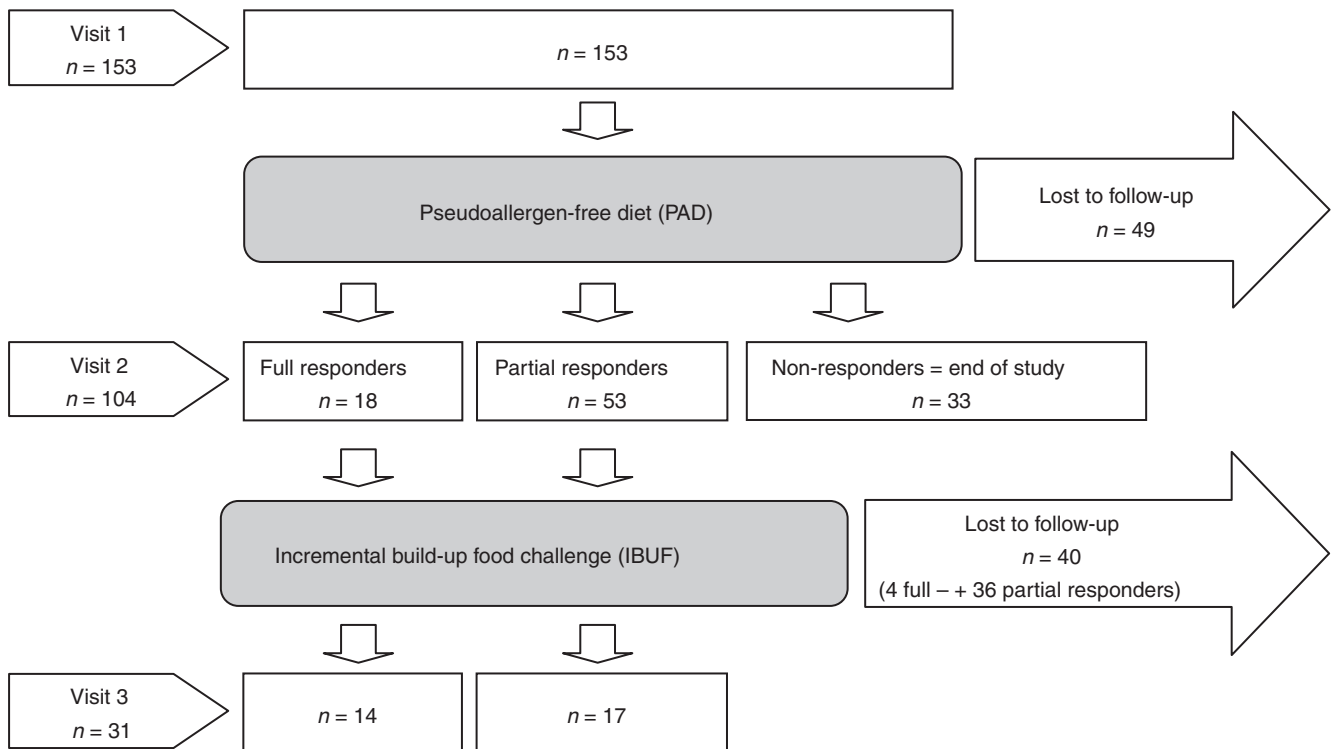


Fig. 1. Study flowchart.

Table 1. Description of the pseudoallergen-free diet (PAD)

	Permitted: pure, natural foods without artificial additives or flavours	Prohibited: all foods containing artificial additives, e.g. convenience food, sweets
Beverages	Pure water, mineral water, coffee, black tea, milk, buttermilk	Herbal, rose hip or fruit teas, water and tea with aroma (e.g. citrus, bergamot, vanillin), flavoured instant coffee
Basic foods	Potatoes, noodles (without egg), rice, pure bread and rolls, flour (not self-raising), rice cakes and unprocessed cereals (e.g. pure corn flakes)	All others: e.g. bread or rolls with herbs, raisins, nuts or oil seeds (e.g. sun flower, pumpkin, flax seeds), potato chips, crisps, cakes, biscuits
Fats	Butter, refined plant oils	All others (e.g. margarine, cold pressed plant oils*)
Milk products	Milk, pure cream, pure buttermilk, pure curd, cream cheese without additives, young gouda (mild)	All others (e.g. fruit yoghurt, cacao, herbal cheese, spread cheese and all other flavoured milk products)
Meat, fish, eggs	Fresh meat without any additives or seasonings (only prepared with salt and oil)	All others: fish, eggs and meat with seasonings or additives
Vegetables	All except those listed as forbidden (e.g. lettuce, carrots, zucchini, cabbage, broccoli)	Tomatoes and tomato products, sweet peppers, olives, spinach, rhubarb, mushrooms, peas, artichokes, onions*
Fruits	None	All kind of fruits including fruit juices, puree, stewed and dried fruits (e.g. raisins)
Herbs, spices	Salt, sugar, pure vinegar to dilute with water (without herbs, balsamic, wine aromas)	All other including chives*, pepper, chilli, herbs, garlic or other seasonings, sweeteners
Sweets, spreads	Honey, sugar beet molasses, (mild gouda)	All other

*Aberrant of the PAD [11].

substances from the literature and was ordered by their occurrence in foods (as described below). Based on the PAD, which do not contain any known pseudoallergens, every new step contains additionally food ingredients, which might be act as pseudoallergens.

- *Step I:* It contained foods, which are not highly suspected to act as pseudoallergens [e.g. organic (bio) eggs, cold-pressed olive oil, fresh sea fish, pears], to increase the compliance for further intervention. These foods also reappear later in a processed manner.

Table 2. Description of the incremental build-up food challenge (IBUF)

Step	Cumulative ingredients	Food selection
I	None	Whole flour bread, onions, garlic, organic (bio) eggs, cold-pressed olive oil, fresh sea fish, pears, watermelon, honey melon
II	Biogenic amines, nitrite, nitrate	Well-maturated cheese without herbs and other food additives such as nitrate, nitrite and beta-carotene: e.g. Emmentaler, Cheddar, Gouda, Camembert, Appenzeller, Edamer; Scombroidae: tuna, mackerel, herring, sardine; pickled cabbage, kohlrabi, spinach, beetroot, avocado, banana, walnut
III	Natural phenolic substances such as <i>p</i> -hydroxy benzoic acid, cumaric acid, salicylic acid, natural flavours, ethereal oils	All fruits, fruit juice, all vegetables (e.g. tomatoes), other nuts, isolated herbs and spices (no instant broth), natural herbal and fruit teas (without any flavouring)
IV	Food additives (without dyes), industrial aromas	Foods are permitted, if no dyes (E100–E199) are added (exception: beta-carotene and riboflavin as natural dyes used for colouring cheese and butter) e.g. instant soup or broth, convenience food, sauces, chips, pizza, nut nougat crème, sweets, soft drinks, chocolate, cacao, all flavoured foods, e.g. teas
V	Food dyes (E100–E199)	Eggs, coloured foods with natural and artificial food dyes, e.g. coated chocolate lenses, bubble gums, drops, bonbons, soft drinks, ice creams, wine gums, custard, gelee dessert
VI	Alcohol	Beer, wine, liquor, spirits

- **Step II:** It contained foods with high amounts of biogenic amines, e.g. tuna, well-maturated cheese, bananas and walnuts [28–32]. We choose biogenic amines at this time-point, because there are foods containing biogenic amines but no other additives or seasonings: pure cheese without herbs, pure tuna in plant oil and salt without other additives. Beta-carotene, nitrite and nitrate are permitted at this step, because these ingredients can be found in many pure natural foods, which have already been consumed within the PAD (e.g. vegetables).
- **Step III:** Natural flavours, aromatic compounds, such as *p*-hydroxyl benzoic or salicylic acid were the main ingredients of this step, e.g. fruits, herbs, tomatoes and isolated spices [21, 33–36]. Up to step III, there are only pure foods with no considerable artificial food additives or flavours. For this reason, the nutritional consulting of the patients is highly important, not using convenience foods such as flavoured teas or broth (containing glutamate).
- **Step IV:** It contained all foods supplemented with different additives (glutamate, sulphite, preservatives, anti-oxidants, flavours), with exception of food dyes [12, 22, 37–43]. There are a lot of convenience foods without any artificial dyes, but containing other additives or seasonings. For this reason, step IV (food additives exceptionally dyes) must be given before step V (food dyes) and after step III (herbs, natural aromatic compounds).
- **Step V:** All coloured food and beverages were permitted and patients were advised to choose preferen-

tial sweets and beverages containing different food dyes (E100–E199) [44, 45]. Furthermore, conventional [non-organic (non-bio)] eggs were now allowed, because hens feed can be supplemented with dyes (canthaxanthin), which can be found in the egg yolk [46].

- **Step VI:** Alcohol was permitted only in the last step, because alcohol has been known to elicit urticaria directly and may lead to increased resorption of possible pseudoallergens [24–26].

For a detailed description of the permitted foods for each IBUF step, refer to Table 2. The adherence to the current order is of high importance, so that suspected foods (containing pseudoallergens) will not be mixed up. There was no restriction within the permitted foods at each step, but the patients were advised to eat high amounts of the step-specific newly introduced foods. If a step was well tolerated during the entire week, all the newly introduced foods were permitted for the consequent steps. In case of recurrence of urticaria or angioedema during a single step, the patients were advised to stop the actual step, go back one step in the food build-up until complete remission of urticaria, skip the incompatible step and go on with the next higher step.

Clinical evaluations

The urticaria severity was determined by urticaria score, proposed by Zuberbier *et al.* [11] as follows: 0 = no symptoms, 1 = mild, 2 = moderate, 3 = intense. Medication

intake was recorded in absolute tablets of antihistamines or milligram prednisolone equivalent. A validated CU quality of life questionnaire (CU-Q₂oL) has been translated into German language and was completed by 113 of 153 patients. This questionnaire contained 23 items, fragmented in six dimensions (pruritus, swelling, impact on life activities, sleep problems, limits and looks). Every item contained a five-point Likert scale as follows: 1 = not at all, 2 = a little, 3 = moderate, 4 = a lot, 5 = very much. The maximal CU-Q₂oL score was 92 points and constitutes the greatest impairment of life quality [47]. By visual analogue scale (VAS), patients were asked about their overall general disturbance due to urticaria symptoms [48, 49]. In this scale, 0 mm meant no disturbance and 50 mm was the maximum. These parameters were evaluated at inclusion (visit 1), after the 5-week-long PAD (visit 2) and after the 6-week-long IBUF (visit 3).

Full responders (FRs) were defined by complete remission after the PAD without intake of antihistamines or corticosteroids within the last 7 days at visit 2. A reduction in the urticaria score by at least one point or a reduction in anti-allergic medication without worsening of urticaria symptoms was determined as partial responder (PR). The primary endpoint of this study was the percentage of FRs, who developed urticaria during at least one step of the IBUF identifying at least one causative food ingredient. The secondary endpoint was the percentage of FRs who were still free of symptoms as assessed by telephone follow-up. Furthermore, we evaluated the outcome of PAD by analysing the reduction in urticaria score, antihistamine intake, the quality of life and the general disturbance due to urticaria by VAS.

Statistical analyses

Demographic and efficacy parameters were given as rates, as mean and standard error of the mean (SEM) values or as median and range, as appropriate. For statistical comparison of demographic data between male and female patients, we used the Mann-Whitney *U*-test. The Wilcoxon test was used for comparison of two time-points within one group. Influencing factors of diet responders were analysed by Fisher's exact chi-square test. *P*-values of 0.05 or less were considered as statistically significant. For statistical calculations, the software package SPSS v. 14.0 (SPSS Inc., Chicago, IL, USA) was used.

Results

Patients' characteristics

In total, 153 patients were included in this study. The male-to-female ratio of 1 : 2 is in accordance with other studies [11, 17, 50]. The mean duration of urticaria was longer than 6 months in 52.3% of patients, and for 47.7% of patients, it was between 6 weeks and 6 months. In our study, females reported a longer history of urticaria than males (*P* = 0.048) and documented a higher impairment in quality of life (*P* = 0.049) at visit 1. At inclusion, 109 of 153 patients were treated with antihistamines (*n* = 93), or oral corticosteroids (*n* = 7) or both (*n* = 9). Demographic data and clinical characteristics are shown in Table 3.

Pseudoallergen-free diet

Out of 153 patients, 104 completed the PAD and 49 were lost to follow-up (Fig. 1). Sixty-eight percent showed an

Table 3. Demographic data and clinical characteristics at inclusion

	All patients	Male	Female	<i>P</i> -value
<i>n</i>	153 (100%)	51 (33%)	102 (67%)	0.001*
Median age, years (range)	41 (10–76)	43 (13–68)	39.5 (10–76)	0.336
Pollinosis	15/153 (9.8%)	5/51 (9.8%)	10/102 (9.8%)	1.000
Asthma	8/153 (5.2%)	1/51 (2.0%)	7/102 (6.9%)	0.201
Atopic dermatitis	5/153 (3.3%)	1/51 (2.0%)	4/102 (3.9%)	0.522
Duration of CU	–	–	–	0.048*
6 weeks to <3 months	22/153 (14.4%)	8/51 (15.7%)	14/102 (13.7%)	
3–6 months	51/153 (33.3%)	23/51 (45.1%)	28/102 (27.5%)	
>6 months	80/153 (52.3%)	20/51 (39.2%)	60/102 (58.8%)	
Antihistamine intake during the last 7 days, mean (SEM) of absolute tablets	102/153 (66.7%) 5.90 (0.53)	32/51 (62.8%) 5.84 (0.99)	70/102 (68.6%) 5.92 (0.63)	0.611
Oral steroid intake (mg prednisolone equivalent) during the last 7 days, mean (SEM)	16/153 (10.5%) 9.69 (3.19%)	3/51 (5.9%) 6.48 (5.01%)	13/102 (12.7%) 11.30 (4.09%)	0.200
Urticaria score, mean (SEM)	2.14 (0.06)	2.10 (0.12)	2.17 (0.08)	0.635
VAS, mean (SEM)	32.10 (1.02)	29.06 (1.98)	33.62 (1.15)	0.078
Total score of CU-Q ₂ oL, mean (SEM); (<i>n</i> = 113)	55.19 (1.38)	50.67 (2.04)	55.19 (1.38)	0.049*

*Statistical significance by Mann-Whitney *U*-test for independent groups (male/female); niveau of significance, α = 0.05.

CU-Q₂oL, chronic urticaria quality of life questionnaire; VAS, visual analogue scale.

improvement in symptoms and quality of life after the PAD, as indicated by a significant reduction in urticaria score, VAS, corticosteroid intake and CU-Q₂oL (each $P < 0.001$).

In total, 18 patients were determined as FRs with an urticaria score of 0 showing a significant decrease in VAS and CU-Q₂oL.

Fifty-three patients were determined as PRs, 8 out of 42 of these patients were able to discontinue their antihistamines after the PAD and six of seven their oral steroids. A significant decrease was seen in mean urticaria score, VAS, steroid intake and mean CU-Q₂oL score.

Thirty-three patients were determined as non-responders (NRs) with a significant increase in urticaria score (2.03 before vs. 2.15 after PAD) and no change in VAS, CU-Q₂oL and/or anti-allergic treatment compared with visit 1 and were excluded from the study (Table 4).

Influence factors on dietary outcome of pseudoallergen-free diet

All demographic parameters were analysed to determine their influence on the dietary response. Neither age, gender, atopic disease, urticaria severity, nor urticaria duration appear to have a significant influence on the dietary outcome (Table 4).

Of note, there was no statistically significant difference in dietary outcome ($P = 0.53$) between patients with short (< 3 months), medium (3–6 months) or long (> 6 months) duration of CU, i.e. 1 FR, 6 PRs, 7 NRs vs. 8 FRs, 17 PRs, 10 NRs vs. 9 FRs, 30 PRs, 16 NRs, respectively.

NRs were taking significant less antihistamine at visit 1 compared with PRs ($P = 0.01$), but during PAD the antihistamine intake and the urticaria score significantly increased in NRs. There was no significant difference between NR and FR or FR and PR in antihistamine intake before starting PAD.

Patients with potentially associated diseases (possible foci), as diagnosed during the initial work-up, have been analysed for an influence on dietary outcome. Only patients with dental foci, as tooth root infection or periodontitis (19 of 52 who were examined), showed an inferior diet response of 42% compared with patients without dental foci 79% ($P = 0.014$). Further statistical analyses showed no influence of sensitization against common allergens, thyroid antibodies, helicobacter pylori or other infections on the dietary outcome (data not shown).

Incremental build-up food challenge

A total of 31 (20%) of all 153 patients completed the IBUF protocol. Out of 71 patients, showing either partial or full response during PAD, 31 completed the IBUF protocol and 40 (36 PRs and 4 FRs) were lost to follow-up (Fig. 1). In total, 12 of the remaining 14 FRs (86%) showed a

Table 4. Clinical outcomes after 5 weeks pseudoallergen-free diet (PAD)

	All patients ($n = 104$)			Full responders ($n = 18$)			Partial responders ($n = 53$)			Non-responders ($n = 33$)		
	Visit 1	Visit 2	P-value	Visit 1	Visit 2	P-value	Visit 1	Visit 2	P-value	Visit 1	Visit 2	P-value
Urticaria score [†]	2.15 (0.08)	1.37 (0.10)	0.000*	2.06 (0.25)	0	0.000*	2.26 (0.10)	1.34 (0.10)	0.000*	2.03 (0.12)	2.15 (0.12)	0.046*
VAS [†]	31.66 (1.27)	19.85 (1.42)	0.000*	29.33 (3.63)	2.06 (0.25)	0.001*	32.34 (1.64)	20.42 (1.61)	0.000*	31.85 (2.30)	28.15 (2.45)	0.074
Antihistamine intake [‡]	5.86 (0.62)	5.55 (0.76)	0.426	5.67 (1.13)	0	0.001*	7.00 (0.91)	5.96 (1.10)	0.238	4.12 (1.09)	7.91 (1.43)	0.000*
<i>n</i>	71	57		13	0		42	34		16	23	
Corticosteroid intake [§]	5.95 (2.83)	0.19 (0.19)	0.012*	5.64 (4.28)	0	0.180	9.76 (5.32)	0.38 (0.38)	0.035*	0	0	1.000
<i>n</i>	9	1		2	0		7	1		0	0	
CU-Q ₂ oL [‡]	55.70 (1.66)	49.14 (1.72)	0.001*	50.00 (4.88)	35.82 (1.86)	0.016*	60.03 (2.27)	53.00 (2.28)	0.012*	50.74 (2.44)	49.42 (3.47)	0.896
<i>n</i>	67	67		11	11		37	37		19	19	

[†]Data shown as means (SEM) during the previous 7 days.

[‡]Absolute tables during the previous 7 days, data shown as means (SEM).

[§]Prednisolone equivalent during the previous 7 days, data shown as means (SEM).

^{||}Subgroup of patients who filled out the quality of life questionnaire (CU-Q₂oL).

*Statistical significance between visit 1 and visit 2 by Wilcoxon test; niveau of significance, $\alpha = 0.05$.

Table 5. Evaluations of the incremental build-up food challenge (IBUF) and long-term follow-up in full responders ($n = 14$)

ID	IBUF						Telephone follow-up			Symptoms during IBUF in agreement with telephone follow-up
	Step I	Step II	Step III	Step IV	Step V	Step VI	Eliciting foods/substances?	Avoidance actually?	Free of symptoms?	
1	+	++	+				Fruits and fruit products	Yes	Yes	Yes
2		++		++			Bananas	Yes	Yes	Yes
3		+	+				Bananas, tomatoes, tuna	Yes	Yes	Yes
4					+		Food dyes and additives	Yes	Yes	Yes
5			+		+		Dates, chocolate and cacao products (high amounts)	Yes	Yes	Yes
6						++	Red wine, fruit and herb liquors	Yes	Yes	Yes
7		+	+				Pears, grapes, nuts, curry	Yes	Yes	Yes
8					+	+	Food dyes and additives, red wine	No*	Yes	Yes
9				+			Salami	No [†]	Yes	Yes
10							Food dyes and additives	Yes [‡]	Yes	No
11							Not found	NA [§]	Yes	NA
12	++	++	++	++	++	++	Not found	NA [¶]	No	NA
13		++		++			Lost to follow-up	NA		
14						+	Lost to follow-up	NA		
<i>n</i>	2	6	5	4	4	4				

+, urticaria; ++, urticaria and antihistamine intake; NA, not applicable.

*For 2 months after IBUF, free of symptoms due to avoidance; then, helicobacter eradication was done and all foods were compatible.

[†]Salami was eaten after the IBUF again, with no development of symptoms, and spontaneous remission should be considered.

[‡]Patient decided to avoid food additives for personal reasons, although no causative food was found during IBUF.

[§]Spontaneous remission likely.

[¶]Questionable compliance.

recurrence of urticaria symptoms during at least one step of the IBUF protocol. Two patients were symptom free during the complete IBUF. Four patients reacted only in one, six in two and one in three different steps. Most of these patients ($n = 6$) developed urticaria and/or angioedema within step II containing biogenic amines and five patients developed symptoms within step III. Per four patients flared in step IV, V and VI and only two in step I (Table 5). The time interval between starting a new IBUF step and the onset of symptoms varied between 1 and 5 days. Symptoms typically lasted between 1 and 6 days within one step, with a high variability between patients and steps. The severity of the experienced symptoms was typically mild to moderate in our urticaria scoring system: non-confluent weals of limited extension and/or up to three deeper swellings each < 10 cm. All data were taken from patient's diary.

All PRs ($n = 17$) showed symptoms during the IBUF, and 16 of them needed antihistamines. Most of the PRs had urticaria symptoms continuously, and it was therefore impossible to identify urticaria-eliciting foods during IBUF protocol. However, the evaluation of the CU-Q₂L questionnaire in PRs (data available of 15 patients) showed a significant increase in quality of life score during IBUF ($P = 0.02$). None of the patients experienced anaphylaxis or severe urticaria/angioedema.

Extrapolating the results of all CU patients, 8% (12/158) showed a primary full response to PAD and a secondary recurrence of symptoms due to IBUF.

Follow-up

After 3 to 24 months of the termination of the IBUF protocol, patients were called and asked if they could still identify one or more eliciting food during the IBUF. Two FRs and four PRs were lost to follow-up. Seventy-five percent of the remaining 12 FRs reported to have found one or more eliciting foods, corresponding with their urticaria. Seven of them (58%) were still free of symptoms at telephone follow-up avoiding the eliciting foods. Seventy-one percent of the patients identified fruit and fruit products, which were represented within step II (banana) and step III as the most incompatible foods. This was followed by food additives and dyes (43%) and alcoholic beverages (29%). The lowest frequency was given for nuts, curry, chocolate/cacao, tomatoes and tuna (Table 5).

Seven of the 17 PRs reported to have identified one or more foods to be responsible for their urticaria symptoms at the telephone follow-up, but these findings were independently of the IBUF outcome. Six patients did not report any eliciting agent or food.

Extrapolating the results of all CU patients, 4% (7/158) were still free of symptoms due to the avoidance of identified foods.

Discussion

This is a pilot study addressing the diagnostic value of a stepwise food provocation (IBUF) in outpatients suffering from CU. All studies so far have focused on oral food challenge tests by administering isolated food additives in capsules without paying attention to individual dislikes or preferences in food choices [5, 6, 11–13, 17, 18, 51, 52]. We tried to address these challenges and our study design taken into consideration: (1) the dose dependency of pseudoallergic reactions, (2) interactions between foods and food ingredients, (3) the individual dietary habits at home, (4) challenge of hitherto unknown pseudoallergens in the patients' individual diet. We used a newly developed, well-defined protocol to expose patients to food containing pseudoallergens (IBUF), to evaluate the recurrence of urticaria.

All patients with complete remission due to PAD (FR) were monitored for recurrence of urticaria during different steps of IBUF. A recurrence of urticaria was observed in 86% of FRs and in all PRs. Comparisons of response rates to pseudoallergens in capsules after a primary elimination diet vary between 19% and 44% [11, 13, 17]. Challenge tests were performed in patients reporting a 'remission', 'improvement' or marked 'improvement' due to an elimination diet. In our study, we give data for patients with a 'complete remission' (completely free of symptoms and no antihistamine or steroid intake) 'due' to a primary elimination diet. Looking at all patients who completed the IBUF protocol (FR and PR), 41% (29/71) showed a recurrence of urticaria. This is in accordance with the findings of Di Lorenzo *et al.* [17], who showed a partial remission in 264 of 838 (31%) CU patients due to a PAD. In addition, 44% (116/264) of these patients showed a positive challenge with mixed food additives. To determine the responsible food additives, all food additives were challenged separately again and only 24 (21%) of the mixed food additive responders showed reproducible symptoms. By extrapolation of these results, it can be determined that 3% of the CU patients can identify an eliciting food additive by primary elimination and secondary challenge procedure. An intention-to-treat-analysis of this study shows that recurrence of CU symptoms during IBUF, after complete remission rate during PAD, can be expected to be 8% ($n = 12$). However, this percentage might be underestimated, because only FRs were evaluated in this analysis.

The comparatively high response rate in our study might be due to the unblinded design, which could lead to a psychological effect increasing the efficacy of IBUF. Most frequent recurrence of symptoms was seen during

step II (6/14) and III (5/14) of the IBUF protocol. This highlights that biogenic amines and natural food ingredients are playing an important role in pseudoallergic reactions as described for tomatoes, spices and citrus aromas [9, 21, 22]. Interestingly, diets with low amounts of biogenic amines (including PAD) seemed to be effective to reduce CU symptoms, but there is no report of a positive 'oral' challenge with histamine causing urticaria, as described in healthy volunteers [53]. The therapeutical effect of a diet low in biogenic amines is thought to be caused by a reduced diamine oxidase activity and subsequently decreased histamine catabolism in CU patients [54, 55].

A deterioration of urticaria was observed in all PRs, except one patient, who took antihistamines daily during the IBUF. Forty-seven percent of the patients suffered continuously from urticaria and 29% took antihistamines during all six steps. For this reason, we assume that this study could not detect a diagnostic value for PRs by means of the IBUF protocol.

To monitor the long-term efficacy, our study design included a telephone follow-up. Eighty-six percent of FRs were available for this follow-up, of those 75% FRs were able to identify incompatible foods in accordance with findings during IBUF and 58% were still free of symptoms due to the recommended dietary restrictions at the time of the telephone interview. These patients clearly identified at least one food or food group which was responsible for their urticaria and they were completely free of symptoms due to avoidance. Patients' statements of incompatible foods in our study were fruits, alcohol, food additives, food dyes, cacao products, nuts, curry, tomatoes and tuna (Table 5). This is in accordance with the findings of Zuberbier [56], who verified food incompatibilities by DBPCFC.

Four of the 12 FRs (33%) were likely to have encountered a spontaneous remission, which is in conformity with the known spontaneous remission rate in CU after 12 months [50, 57, 58]. Patient number 8 remained free of symptoms for 2 months after IBUF, avoiding food dyes and alcohol. Then, helicobacter eradication was done, and at the time of telephone follow-up, all foods were compatible. Patient number 9 showed recurrence of urticaria during step IV during IBUF (due to a special kind of salami), demonstrating a positive test result for this specific food during IBUF. Although at the telephone follow-up, he said to now tolerate the above-mentioned salami hinting at spontaneous remission. Patient number 10 decided to avoid food additives for personal reasons, although no causative food was found during IBUF. Patient number 11 was completely free of symptoms during the entire IBUF and the follow-up period also, indicating a spontaneous remission even before the IBUF protocol started.

The main advantage of challenge tests with capsules is the well-defined amount of food additives and the option

of DBPCFC design, which enable a better standardization and comparability of study protocols. However, this design has several drawbacks as well the selection of additives is discretionary, and most importantly, the amounts are often not in accordance with a realistic intake [38, 59]. The maximal possible salicylate intake has been estimated to be 6 mg per day for a salicylate-rich vegetarian diet [60], but challenge tests are done with total doses of 100 mg [11, 12] which is equivalent to 2 kg red currents or 7 kg strawberries, representing foods with a natural high amount of salicylic acid [32]. In contrast, oral challenges with sodium glutamate are often done at low concentrations (200 mg) compared with the daily intake of 5–12 g in European countries [11, 61]. Hence, outcomes of oral capsule challenges with food additives in excessive or too low amounts should be interpreted with caution and alternatives are needed. Another important factor that we address with our study is the rapid change in use of artificial additives by the food industry. Within a longitudinal study, collecting detailed data on diet in children and adolescents, the analysis of consumed convenience foods showed that 20% of food ingredients are flavours or additives, whereas flavours and taste enhancers are on the top [27]. Furthermore, the compositions of added food flavours are highly confidential and supply is very time-consuming. An ideal food challenge should be focussed more on natural food ingredients and flavours, instead of concentrating on the known food dyes and preservatives, as common challenge protocols do.

The high responsiveness to PAD (68%) observed in this study is in accordance with other studies dealing with PAD [5, 11–13, 16] and exceeded the reported spontaneous remission in CU [50, 57, 58]. Considering the difficulties in finding a suitable placebo for a diet in an outpatient manner, a placebo effect cannot be excluded. However, an additional dietary placebo effect could never be ruled out even when regular and placebo diet regimes are compared. Other dietary interventions have been done for duration between 10 and 21 days, whereas anti-allergic medication was prohibited [5, 6, 11, 12]. To obtain a realistic CU population, consisting of patients with and without anti-allergic treatment, patients were permitted to use antihistamines and corticosteroids at the minimum dose needed to control urticaria. For this reason, the duration of PAD was determined to be a period of 5 weeks, to allow all patients the discontinuation of their oral anti-allergic treatment.

There is little information about the drop-out rates during elimination diets in CU. Pigatto and Valsecchi [13] reported that 38% of patients were lost to follow-up during a PAD. This is in accordance with our results. When the patients were informed about the vast amount of possible causes for their urticaria symptoms, they felt often too overwhelmed and frustrated to comply with the rigid protocol of PAD. The diet was consequently not

appropriately followed or even stopped after a few days. We felt that this is one of the major reasons for the rather high drop out rate of 32% during PAD. We have no information about reasons for drop-out (non-compliance, remission of urticaria or lack of efficacy). In the daily work, many patients are seen favouring a drug therapy instead of following a special dietary protocol. Patients with a good or very good response to a diet might also decide to go on further with an effective protocol, but are failing to meet the scheduled follow-up visits. A telephone follow-up interview could give the reasons for drop-outs and should be integrated in future clinical trials.

The high drop-out rate in PRs (68%) during IBUF can be explained due to lack of efficacy in primary PRs. These patients did not reach a complete remission after 5 weeks of PAD and flared up as expected during IBUF. In contrast to the PRs, the drop-out rate of FRs was relatively low (22%), indicating a continuous motivation to perform the IBUF for this patient subgroup (Fig. 1).

To our knowledge, the quality of life was evaluated for the first time in CU patients, before and after a dietary intervention. There was a significant amelioration of all responders during the PAD, which remained stable during IBUF. The data of CU-Q₂oL in FRs showed no significant change in life quality during IBUF. The questionnaire used (CU-Q₂oL) comprised two questions about impairment on dietary restrictions and eating habits (data not shown). As expected, there was a significant impairment by the PAD due to these items, but the improvement in total quality of life in all responders remained stable and significant (Table 4). Hence, to be symptom free in CU patients was of greater importance than the decrease in quality of life due to dietary restrictions.

This is a pilot study encountering its usual limitations. We used an open and uncontrolled design to get initial information about this newly developed protocol considering the dietary habits under real life situations of the patients.

We recommend IBUF to identify individual eliciting foods in 'high diet sensitive' CU patients. A 'full' response to the PAD (elimination diet) was the precondition to enter IBUF. The here-given IBUF protocol can help CU patients to identify their individual 'specific' pseudoallergens that are responsible for their symptoms. As shown in the telephone follow-up, these patients reported full remission of their symptoms due to an avoidance of the identified foods. For these patients also, antihistamines were not necessary any longer. Therefore, our IBUF protocol can help CU patients to achieve a long-lasting remission of their symptoms by an easy-to-follow diet protocol. Nevertheless, due to the pilot character of our study, it is necessary to verify the data in randomized and well-controlled studies. While data on effectiveness of PAD are convincing, the standard capsule provocation test should be reconsidered.

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References

- Kulthanan K, Jiamton S, Thumpimukvatana N, Pinkaew S. Chronic idiopathic urticaria: prevalence and clinical course. *J Dermatol* 2007; **34**:294–301.
- Grattan CEH, Sabroe RA, Greaves MW. Chronic urticaria. *J Am Acad Dermatol* 2002; **46**:645–57.
- Powell RJ, Du Toit GL, Siddique N *et al*. BSACI guidelines for the management of chronic urticaria and angio-oedema. *Clin Exp Allergy* 2007; **37**:631–50.
- Doeglas HM. Dietary treatment of patients with chronic urticaria and intolerance to aspirin and food additives. *Dermatologica* 1977; **154**:308–10.
- Gibson A, Clancy R. Management of chronic idiopathic urticaria by the identification and exclusion of dietary factors. *Clin Allergy* 1980; **10**:699–704.
- Rudzki E, Czubalski K, Grzywa Z. Detection of urticaria with food additives intolerance by means of diet. *Dermatologica* 1980; **161**:57–62.
- Verschave A, Stevens E, Degreef H. Pseudoallergen-free diet in chronic urticaria. *Dermatologica* 1983; **167**:256–9.
- Malanin G, Kalimo K. The results of skin testing with food additives and the effect of an elimination diet in chronic and recurrent urticaria and recurrent angioedema. *Clin Exp Allergy* 1989; **19**:539–43.
- Wantke F, Gotz M, Jarisch R. Histamine-free diet. *Hautarzt* 1993; **44**:512–6.
- Stoneham MD, Young E, Wilkinson JD. Dietary manipulation of fat in chronic urticaria. *J Dermatol Treat* 1993; **4**:183–5.
- Zuberbier T, Chantraine-Hess S, Hartmann K, Czarnetzki BM. Pseudoallergen-free diet in the treatment of chronic urticaria. A prospective study. *Acta Derm Venereol* 1995; **75**:484–7.
- Ehlers I, Niggemann B, Binder C, Zuberbier T. Role of nonallergic hypersensitivity reactions in children with chronic urticaria. *Allergy* 1998; **53**:1074–7.
- Pigatto PD, Valsecchi RH. Chronic urticaria: a mystery. *Allergy* 2000; **55**:306–8.
- King W, McCargar L, Joneja JM, Barr SI. Benefits of a histamine-reducing diet for some patients with chronic urticaria and angioedema. *Can J Diet Pract Res* 2000; **61**:24–6.
- Guida B, De Martino CD, De Martino SD *et al*. Histamine plasma levels and elimination diet in chronic idiopathic urticaria. *Eur J Clin Nutr* 2000; **54**:155–8.
- Buhner S, Reese I, Kuehl F, Lochs H, Zuberbier T. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. *Allergy* 2004; **59**:1118–23.
- Di Lorenzo G, Pacor ML, Mansueto P *et al*. Food-additive-induced urticaria: a survey of 838 patients with recurrent chronic idiopathic urticaria. *Int Arch Allergy Immunol* 2005; **138**:235–42.
- Supramaniam G, Warner JO. Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet* 1986; **2**:907–9.
- Miller R, White LW, Schwartz HJ. Case of episodic urticaria due to saccharin ingestion. *J Allergy Clin Immunol* 1974; **53**:240–2.
- Young E. Prevalence of intolerance to food additives. *Environ Toxicol Pharmacol* 1997; **4**:111–4.
- Zuberbier T, Pfrommer C, Specht K *et al*. Aromatic components of food as novel eliciting factors of pseudoallergic reactions in chronic urticaria. *J Allergy Clin Immunol* 2002; **109**:343–8.
- Fuglsang G, Madsen C, Halken S, Jorgensen M, Ostergaard PA, Osterballe O. Adverse reactions to food-additives in children with atopic symptoms. *Allergy* 1994; **49**:31–7.
- Guerra L, Rogkakou A, Massacane P *et al*. Role of contact sensitization in chronic urticaria. *J Am Acad Dermatol* 2007; **56**:88–90.
- Emonet S, Hogendijk S, Voegeli J, Eigenmann PA, Roux N, Hauser C. Ethanol-induced urticaria: elevated tryptase levels after double-blind, placebo-controlled challenge. *Dermatology* 1998; **197**:181–2.
- Karvonen J, Hannuksela M. Urticaria from alcoholic beverages. *Acta Allergol* 1976; **31**:167–70.
- Ehlers I, Hipler UC, Zuberbier T, Worm M. Ethanol as a cause of hypersensitivity reactions to alcoholic beverages. *Clin Exp Allergy* 2002; **32**:1231–5.
- Alexy U, Sichert-Hellert W, Rode T, Kersting M. Convenience food in the diet of children and adolescents: consumption and composition. *Br J Nutr* 2008; **99**:345–51.
- Latorre-Moratalla ML, Veciana-Nogues T, Bover-Cid S *et al*. Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chem* 2008; **107**:912–21.
- Contreras M, Izquierdo P, Allara M, Garcia A, Torres G, Cespedes E. Biogenic amines determination in ripened cheeses. *Rev Cient (Maracaibo)* 2007; **17**:89–95.
- Fernandez M, Linares DM, del Rio B, Ladero V, Alvarez MA. HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms. *J Dairy Res* 2007; **74**:276–82.
- Eliassen KA, Reistad R, Risoen U, Ronning HF. Dietary polyamines. *Food Chem* 2002; **78**:273–80.
- Souci SW, Fachmann W, Kraut H. *Food composition and nutritional tables*. 5th rev. and completed ed. Edn. Stuttgart: Medpharm Scientific Publishers, 1994.
- Zadernowski R, Naczki M, Nesterowicz J. Phenolic acid profiles in some small berries. *J Agric Food Chem* 2005; **53**:2118–24.
- Tomas-Barberan FA, Clifford MN. Dietary hydroxybenzoic acid derivatives – nature, occurrence and dietary burden. *J Sci Food Agric* 2000; **80**:1024–32.
- Venema DP, Hollman PCH, Janssen KPLT, Katan MB. Determination of acetylsalicylic acid and salicylic acid in foods, using HPLC with fluorescence detection. *J Agric Food Chem* 1996; **44**:1762–7.
- Pfrommer C, Bastl R, Vieths S, Ehlers I, Henz BM, Zuberbier T. Characterization of naturally occurring pseudoallergens causing chronic urticaria. *J Allergy Clin Immunol* 1996; **97**:367.
- Populin T, Moret S, Truant S, Conte LS. A survey on the presence of free glutamic acid in foodstuffs, with and without added monosodium glutamate. *Food Chem* 2007; **104**:1712–7.

- 38 Ishiwata H, Nishijima M, Fukasawa Y. Estimation of inorganic food additive (nitrite, nitrate and sulfur dioxide), antioxidant (BHA and BHT), processing agent (propylene glycol) and sweetener (sodium saccharin) concentrations in foods and their daily intake based on official inspection results in Japan in fiscal year 1998. *Shokuhin Eiseigaku Zasshi* 2003; **44**:132–43.
- 39 Burini G, Damiani P. Determination of sorbic acid in margarine and butter by high-performance liquid-chromatography with fluorescence detection. *J Chromatogr* 1991; **543**:69–80.
- 40 Kurek M, Grubska-Suchanek E. Challenge tests with food additives and aspirin in the diagnosis of chronic urticaria. *Rev Fr Allergol Immunol Clin* 2001; **41**:463–9.
- 41 Belchiernandez J, Floridolopez JF, Estradarodriguez JL, Martinezalzamora F, Lopezserano C, Ojedacasas JA. Sulfite-induced urticaria. *Ann Allergy* 1993; **71**:230–2.
- 42 Goodman DL, McDonnell JT, Nelson HS, Vaughan TR, Weber RW. Chronic urticaria exacerbated by the antioxidant food preservatives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *J Allergy Clin Immunol* 1990; **86**:570–5.
- 43 Kulczycki A. Aspartame-induced urticaria. *Ann Intern Med* 1986; **104**:207–8.
- 44 Böhm M, Bunselmeyer B, Luger TA, Brehler R. Food intolerance due to wine gums: identification of copper chlorophyll (E141) as a possible pseudoallergen. *J Allergy Clin Immunol* 2001; **107**:393–4.
- 45 Huang HY, Shih YC, Chen YC. Determining eight colorants in milk beverages by capillary electrophoresis. *J Chromatogr A* 2002; **959**:317–25.
- 46 Grashorn MA, Steinberg W. Deposition rates of canthaxanthin in egg yolks. *Eur Poult Sci* 2002; **66**:258–62.
- 47 Baiardini I, Pasquali M, Braidò F *et al.* A new tool to evaluate the impact of chronic urticaria on quality of life: chronic urticaria quality of life questionnaire (CU-Q(2)oL). *Allergy* 2005; **60**:1073–8.
- 48 Huskisson EC. Measurement of pain. *Lancet* 1974; **2**:1127–31.
- 49 Engin B, Uguz F, Yilmaz E, Ozdemir M, Mevlitoglu I. The levels of depression, anxiety and quality of life in patients with chronic idiopathic urticaria. *J Eur Acad Dermatol Venereol* 2008; **22**:36–40.
- 50 Toubi E, Kessel A, Avshovich N *et al.* Clinical and laboratory parameters in predicting chronic urticaria duration: a prospective study of 139 patients. *Allergy* 2004; **59**:869–73.
- 51 Genton C, Frei PC, Pecoud A. Value of oral provocation tests to aspirin and food additives in the routine investigation of asthma and chronic urticaria. *J Allergy Clin Immunol* 1985; **76**:40–5.
- 52 Ros AM, Juhlin L, Michaelsson G. A follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes. *Br J Dermatol* 1976; **95**:19–24.
- 53 Wohrl S, Hemmer W, Focke M, Rappersberger K, Jarisch R. Histamine intolerance-like symptoms in healthy volunteers after oral provocation with liquid histamine. *Allergy Asthma Proc* 2004; **25**:305–11.
- 54 Lessof MH, Gant V, Hinuma K, Murphy GM, Dowling RH. Recurrent urticaria and reduced diamine oxidase activity. *Clin Exp Allergy* 1990; **20**:373–6.
- 55 Pollock I, Murdoch RD, Lessof MH. Plasma histamine and clinical tolerance to infused histamine in normal, atopic and urticarial subjects. *Agents Actions* 1991; **32**:359–65.
- 56 Zuberbier T, Edenharter G, Worm M *et al.* Prevalence of adverse reactions to food in Germany – a population study. *Allergy* 2004; **59**:338–45.
- 57 Henz BM, Zuberbier T. Most chronic urticaria is food-dependent, and not idiopathic. *Exp Dermatol* 1998; **7**:139–42.
- 58 Kozel MMA, Mekkes JR, Bossuyt PMM, Bos JD. Natural course of physical and chronic urticaria and angioedema in 220 patients. *J Am Acad Dermatol* 2001; **45**:387–91.
- 59 Steering Group on Chemical Aspects of Food Surveillance., Great Britain Ministry of Agriculture FaF. *Dietary intake of food additives in the UK: initial surveillance; the thirty-seventh report of the Steering Group on Chemical Aspects of Food Surveillance*. London: HMSO, 1993.
- 60 Janssen PL, Hollman PC, Reichman E, Venema DP, vanStaveren WA, Katan MB. Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low. *Am J Clin Nutr* 1996; **64**:743–7.
- 61 Beyreuther K, Biesalski HK, Fernstrom JD *et al.* Consensus meeting: monosodium glutamate – an update. *Eur J Clin Nutr* 2007; **61**:304–13.