

Short communication

The effect of dry heat on mite, cat, and dog allergens

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Background Various techniques have been tried in an attempt to reduce allergen levels in homes. This study investigated the effect of dry heat on mite, cat, and dog allergens.

Methods Samples (50 mg) of *Dermatophagoides pteronyssinus* and *D. farinae* cultures, and of house dust rich in the major cat and dog allergens Fel d 1 and Can f 1 were heated for 5, 10, 15, 30, and 60 min at 60°, 80°, 100°, 120°, and 140°C. Control samples remained at room temperature. Extracts were assayed with the appropriate two-site mono- or mono/polyclonal sandwich ELISA.

Results For Der p 1, the breakdown was proportional to temperature and heating time; after 30 min at 120°C, allergen levels were reduced to <1% of control. Der p 2 was more heat stable, requiring 140°C for 30–60 min to achieve >99% reduction. *D. farinae* groups 1 and 2 allergens showed results similar to those obtained with *D. pteronyssinus*. In contrast, Can f 1 and Fel d 1 were considerably more thermostable, with 50% and 70%, respectively, of allergen remaining after 60 min at 140°C.

Conclusions The effect of dry heat on allergens increased with increasing time and temperature, cat and dog allergens demonstrating greater heat resistance than mite allergens. Dry heating methods may represent an alternative technique for removal of mite allergens; however, the greater stability of Fel d 1 and Can f 1 suggests that this procedure may not be appropriate for pet allergens.

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Allergy to house-dust mites and domestic pets is a major cause of ill health in the UK and around the world (1). The avoidance of exposure to these domestic allergens has been suggested as the primary objective in the clinical management of patients sensitized to these allergens (1, 2). A number of studies have been conducted recently, describing both physical and chemical approaches undertaken in the pursuit of reduction of allergen levels, with varying degrees of success (reviewed by Custovic et al. [2]). This study investigated the effect of dry heat on house-dust-mite, cat, and dog allergens.

Material and methods

Samples (50 mg) of *Dermatophagoides pteronyssinus* and *D. farinae* culture, and of house dust rich in

the major cat and dog allergens Fel d 1 and Can f 1 (316 and 260 µg/g dust respectively), were weighed into test tubes. These were heated in an oven (WTB Binder ovens, Germany) for 5, 10, 15, 30, and 60 min at 60°, 80°, 100°, 120°, and 140°C, respectively. Control samples were left at room temperature. All experiments were repeated on three separate occasions.

Samples were extracted by rotation in phosphate-buffered saline containing 0.1% Tween 20 for 2 h at room temperature, and then centrifuged at 1200 g for 20 min at 4°C. The supernatants were stored at –30°C. The levels of group 1 (Der p 1 and Der f 1) and group 2 house-dust-mite allergens, major cat allergen (Fel d 1), and major dog allergen (Can f 1) were determined by the appropriate two-site monoclonal or mono/polyclonal sandwich ELISAs (3–6).

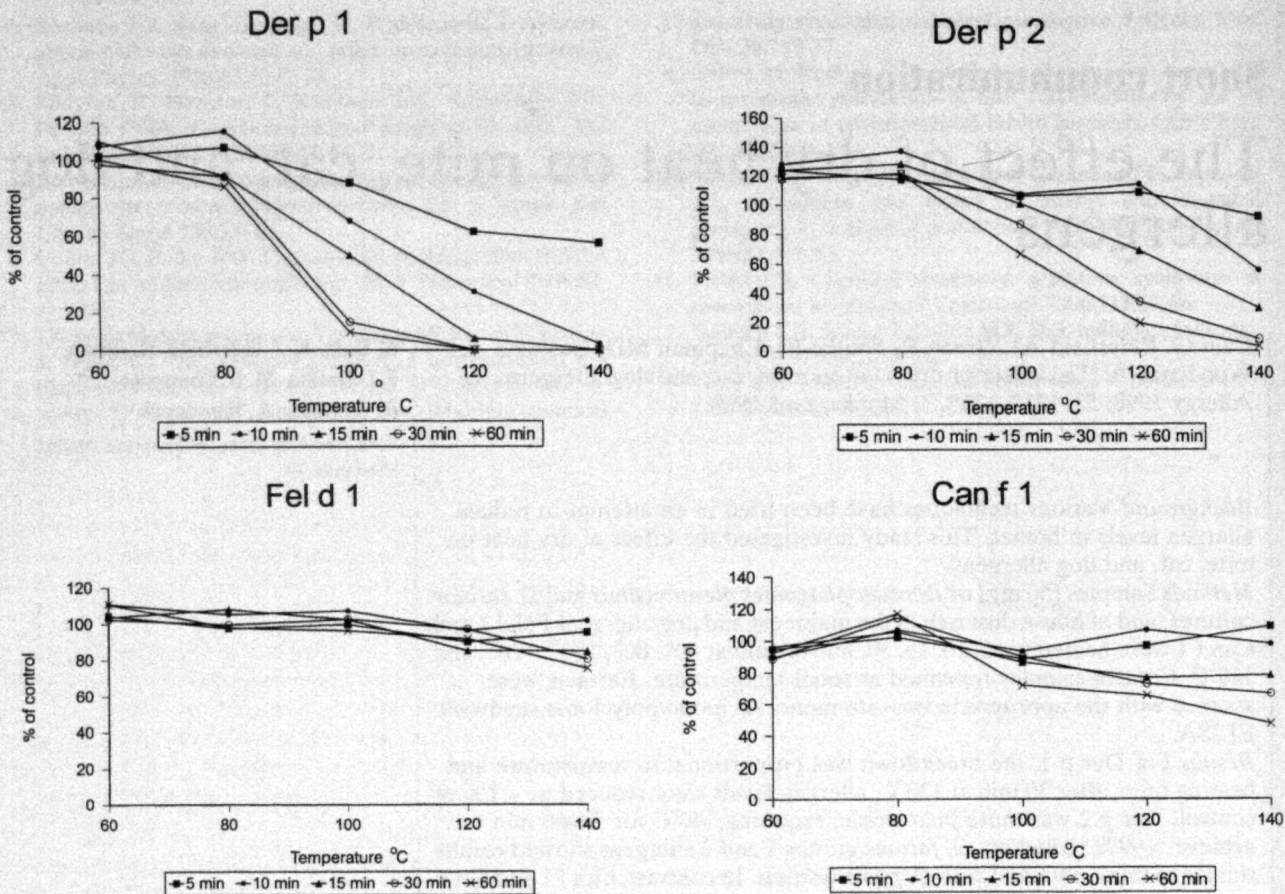


Fig. 1. Effect of dry heat on groups 1 and 2 house-dust-mite and group 1 cat and dog allergens.

Results

The effect of different temperatures and heating times on house-dust-mite, cat, and dog allergen levels is presented in Table 1 and Fig. 1. Der p 1 levels fell with increasing temperature and heating time. Appreciable reductions were observed after heating to 100°C, and the levels dropped to <1% of control after 30-min exposure to 120°C. Der p 2 was more heat stable, requiring 60 min at 140°C to achieve a reduction comparable to that observed for Der p 1 after 30 min at 120°C. *D. farinae* group 1 and group 2 allergens showed similar results to *D. pteronyssinus*, although they appeared slightly less resistant to heat.

In contrast, Can f 1 and Fel d 1 were found to be considerably more thermostable, with very little reduction in allergen levels occurring below 140°C. Even after 60-min exposure to this temperature, levels of Can f 1 and Fel d 1 were 50% and 70% of controls, respectively. Interestingly, in all cases, measured allergen levels increased slightly before decreasing.

Discussion

Changes in temperature have been previously used in an attempt to reduce allergen levels. De Boer suggested autoclaving (110°C for 10 min) as the method of choice against house-dust mites, as this procedure destroys Der p 1, Der p 2, and mite habitat suitability in rugs (7). Freezing with liquid nitrogen has been shown to kill house-dust mites, but has no effect on mite allergens (8).

Lombardero et al. (9) have previously reported that when mite allergens are heated in aqueous solution, there is a good correlation between epitope loss and skin test response, with a 3–4 log reduction in immediate skin test response from native to heated (100°C, 5 min) Der f 1. It was also reported that when group 1 allergens are heated and subsequently analysed by SDS-PAGE, no autolytic digestion of these molecules occurs. We have observed that detectable mite-allergen levels were greatly reduced by exposure to high temperatures, and that group 2 allergens were more thermostable than group 1. This compares favour-

Table 1. Effect of dry heat on house-dust-mite, cat, and dog allergens (numbers represent percentages of control values)

	Temperature	5 min	10 min	15 min	30 min	60 min
Der p 1	60°C	102.2	107.3	109.9	97.9	100.6
	80°C	106.7	115.3	91.7	91.2	85.9
	100°C	88.2	68.1	50.3	15.1	9.5
	120°C	62.1	30.8	6.1	<1	<1
	140°C	56.2	3.21	<1	<1	<1
Der p 2	60°C	124	128.1	123.7	117.9	118.5
	80°C	117.4	137	128.2	121.4	121
	100°C	105.7	107.1	100.6	86.6	66.5
	120°C	108.4	113.8	68.7	34.1	19.2
	140°C	91.9	55.8	29.8	8.1	3.2
Der f 1	60°C	106	112	97	94	92
	80°C	104	115	66	31	12
	100°C	106	12	3	2	<1
	120°C	7	3	<1	<1	<1
	140°C	1	<1	<1	<1	<1
Der f 2	60°C	110	105	134	107	102
	80°C	114	89	134	67	40
	100°C	114	68	46	13	8
	120°C	53	21	14	<1	<1
	140°C	21	2	<1	<1	<1
Can f 1	60°C	94.6	89.2	95.9	88.5	92.4
	80°C	101.9	106.5	105.4	114.2	116.6
	100°C	88.4	93.15	86.4	89.7	71.7
	120°C	96.7	106.5	76.4	72.7	62.25
	140°C	108.9	97.7	77.9	66.3	47.3
Fel d 1	60°C	104.5	111	103.8	102.6	111
	80°C	97.6	105.3	108.2	99.7	98
	100°C	99.8	107.3	102.5	102.8	96.4
	120°C	90.1	96.4	85.2	97.1	91.7
	140°C	95.4	101.8	84.9	80.5	75.4

ably with the findings of Lombardero et al. (9), which suggest that group 1 allergens are conformational, while the allergenicity of Der p 2 and Der f 2 may be explained by the primary structure of the epitope. It is possible, therefore, that dry heat could be used to reduce levels of mite allergens. However, given the greater heat stability of Can f 1 and Fel d 1, it would seem inappropriate to employ this method for pet allergens.

House-dust-mite, cat, and dog allergen levels were determined with monoclonal antibodies. However, as human IgE may recognize different epitopes, the current study does not demonstrate that the thermostable and thermosensitive epitopes recognized by the monoclonal antibodies are the same as those recognized by the IgE. Further studies (e.g., by RAST inhibition) are needed to determine whether the denaturation by heat occurs in the epitopes reacting with human IgE.

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