

Short communication

Heat treatment modifies the allergenicity of beef and bovine serum albumin

Fiocchi A, Restani P, Riva E, Mirri GP, Santini I, Bernardo L, Galli CL. Heat treatment modifies the allergenicity of beef and bovine serum albumin.

Allergy 1998; 53: 798–802. © Munksgaard 1998.

The effect of heat on the allergenicity of beef and bovine serum albumin was investigated among 10 toddlers skin prick test (SPT)-positive to raw and cooked beef. The meat-allergy diagnosis was confirmed during double-blind, placebo-controlled food challenge (DBPCFC) with 180 g of beef cooked for 5 min at 100°C. SPT with homogenized and freeze-dried beef, and heated and unheated bovine serum albumin were performed. Both heated and unheated bovine serum albumin, homogenized beef, and freeze-dried beef were used in trial DBPCFC. All children were SPT-positive to unheated bovine serum albumin. Seven were positive to heated bovine serum albumin, one to freeze-dried beef, and none to homogenized beef. DBPCFCs were negative for homogenized beef and freeze-dried beef, positive for unheated bovine serum albumin in five patients, and positive for heated albumin in four children. We conclude that heating reduces sensitization to beef and bovine serum albumin but does not abolish reactivity to albumin under home conditions. However, industrially heat-treated and sterilized homogenized beef and freeze-dried beef may be suitable substitutes in beef-allergic children's diets.

The prevalence of clinical sensitivity to beef is low during childhood (1–4), although the condition has been found in populations of atopic children (18/113 or 15.9% positive at skin prick test [SPT]; diagnosis confirmed during double-blind, placebo-controlled food challenge [DBPCFC] in 2/18 cases) (4) and of children with atopic dermatitis (AD) and cow's milk allergy (2/15 or 13% confirmed by open diagnostic challenge) (5). Recently, a study of 184 children found 12 patients intolerant of fresh beef during DBPCFC (6). Beef allergy was also diagnosed (but using an unstated challenge dose of beef from an infant food preparation) in 11/335 children with atopy, eight of whom had positive challenge with beef, and three of whom later developed symptoms during open beef challenge, although testing negative at diagnostic challenge (7). The immune and enzymatic mechanisms of clinical reactivity to bovine proteins remain to be elucidated. The reactivity/allergenicity of bovine serum albumin (BSA) has also been the focus of studies of children with atopy (6) and with allergy to cow's milk (8). The effects of industrial processing (9) and enzymatic

digestion (9, 10) on allergenicity have also been evaluated. As a minority of beef-allergic children are sensitized at SPT using homogenized beef (HB) and freeze-dried beef (FDB) (9), we investigated the use of these heat-processed beef proteins in a population of children with a history of meat allergy. The effect of heating on sensitization and reactivity to both heated and unheated BSA was investigated during DBPCFC by the protocol described elsewhere (6). Our results diverge from an earlier study (7) in which three patients developed symptoms after open challenge with fresh beef, although tolerant of beef during diagnostic challenge. However, different protocols and the limited size of both series could account for this discrepancy.

Material and methods

Patients

In 1995, we surveyed (6, 9) 12 consecutive patients presenting with AD and diagnosed hypersensitive

**A. Fiocchi¹, P. Restani², E. Riva¹,
G. P. Mirri¹, I. Santini¹, L. Bernardo¹,
C. L. Galli²**

¹Department of Paediatrics of the University of Milan Medical School at the San Paolo Biomedical Institute, Milan; ²Laboratory of Toxicology of the Institute of Pharmacology of the University of Milan, Milan, Italy

Key words: beef allergy; bovine serum albumin; double-blind, placebo-controlled food challenge; meat allergy.

Alessandro Fiocchi, MD
Department of Paediatrics
San Paolo Biomedical Institute
8, via Di Rudini
Milan 20142
Italy

Accepted for publication 12 March 1998

to meat at open challenge between October 1991 and February 1992. Recalled to confirm this diagnosis, these children underwent DBPCFC patterned on reported home conditions with beef, with turkey as placebo, after negative SPT between January and June 1995. All children were asymptomatic when diagnostic DBPCFC were performed, but six were not rechallenged because they were reported to be tolerant of beef. Four patients tested negative at DBPCFC and two were still allergic to beef. Another group of 13 children reported immediate-onset reactions: asthma ($n=1$), allergic rhinitis and asthma ($n=3$), lip edema and coughing ($n=1$), urticaria ($n=6$), and allergic rhinitis and urticaria ($n=2$); they underwent DBPCFC with beef by the same protocol. An immediate-onset hyperreaction to beef developed in eight children. (Prior informed parental consent was on file.) Ten patients confirmed allergic to beef (four girls and six boys, aged 1.25–4.41 years) were thus recruited and recalled. They underwent SPT and DBPCFC according to the following protocol after institutional ethical review.

SPT

Patients underwent all cutaneous tests during the same visit. Wheal diameters were read through a clear plastic caliper disk scaled in tenths of millimeters under $\times 100$ magnification. Volumes of 10 mg/ml histamine phosphate in 50% glycosaline and glycosaline on its own were used as positive and negative controls, respectively. A wheal wider by at least 3 mm than negative controls up to 30 min after pricking defined positivity. The beef commercial extract (BCE) used consisted of beef from skeletal muscle defatted in ether and acetone and extracted into 50% glycerol at 1 mg/ml concentration (Bayer Pharmaceutical Allergy Products, Spokane, WA, USA, personal communication). Needles loaded with unheated and heated HB and FDB samples were used in skin testing (11). The HB (Plada, Milan, Italy) had been steam-cooked in two (103°C and 98°C) cycles of 20 min each and sterilized at 122°C for 40 min. The FDB (Plada, Milan, Italy) had undergone heat treatment at 100°C for 20 min before sterilization at 121°C and lyophilization at –35°C for 21 h. Both HB and FDB were ground and extracted into 50% glycerol at 1:10 w/v concentration for test purposes. The allergen solution used was suspended in 50% glycerol at a final concentration of 20 mg/ml. The essentially globulin-free BSA sample (Sigma Chemicals, Milan, Italy) was obtained at the highest purification level available (99%). Both the unheated and heated BSA samples (100°C for 5 min) were solubilized in 50% glycerol at 1 mg/ml.

The identity and absence of other proteins were confirmed by electrophoresis.

SDS–PAGE and laser densitometry

A sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE) run was carried out on a gradient gel. The gradient running gel was composed of 9–19% acrylamide, 0.08–0.17% bis-acrylamide, 0.36 M tri-(hydroxymethyl) amino-methane (tris)-HCl, pH 8.8; glycerol 35%; SDS 0.1%; ammonium persulfate 0.02%; and 0.15% 5-*N,N,N,N'*-tetramethylene diamine (TEMED) (12). Bromophenol blue was added to the gel as a run marker. The stacking gel included acrylamide 3.5%, bis-acrylamide 0.09%, tris-HCl 0.125 M, pH 6.8; SDS 0.01%; ammonium persulfate 0.02%; and TEMED 0.15%. BSA and the protein samples were suspended in a sample buffer 0.25 M tris-HCl buffer, pH 6.8, containing glycerol 7.5%, SDS 2%, and β -mercaptoethanol 5%, and heated for 10 min at 100°C. After the samples were loaded onto the gel, the run was performed at 90 V. The gels were dyed with Coomassie brilliant blue G-250 and probed with a laser densitometer. BSA was added by specified increments into the meat samples before their loading onto the gel. Densitometries were processed with the LKD 2400 Gel Scan XL software package. These results were used in order to determine the total BSA challenge doses.

DBPCFC

The largest portion (180 g) and longest cooking time (5 min) on record were used to set both diagnostic and study challenge doses (13) in order to “reproduce the conditions reported by the patient in the history” (14). Steaming was preferred to grilling in the skillet in order to incorporate cooking juices. Mince patties 1 cm in thickness were thus heated for 5 min until the core temperature reached 98°C on a roast thermometer and all the pink meat had turned brown. The DBPCFC with 150 g HB and 60 g FDB ($\cong$ 180 g raw beef) was administered on separate days. Laser densitometry found 90 mg of BSA in 180 g of raw beef, and four doubling doses were thus calculated to deliver a total of 90 mg of BSA. The placebos used with the challenge beef under various forms were 150 g of homogenized turkey and 60 g of freeze-dried turkey preparations. Either lactose as placebo or pear juice (additive-free except for ascorbic acid) as masking food was used after a negative SPT for pear juice in the challenge with unheated BSA. Children who developed symptoms during the unheated BSA challenge were recalled 3–7 days

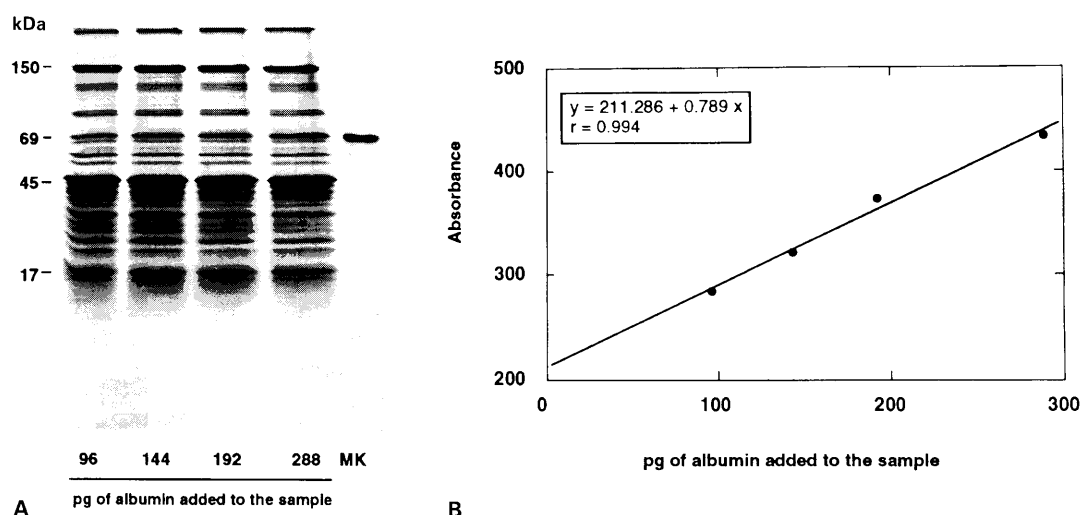


Fig. 1. SDS-PAGE (A) of beef samples with standard addition of serum albumin and linear regression (B) obtained by laser densitometry elaboration on same gel. MK: purified bovine serum albumin.

later and underwent DBPCFC with heated BSA under the same protocol.

Statistical analysis

Wheal diameters were compared to generate descriptive statistics. Results from a single visit were used to allow for inter-/intraindividual differences in priming different responses to several kinds of allergens at different times in the same subject. Spearman's rho was calculated with wheal diameters as outcome variables in the correlation coefficient equation. Linear regression analysis of BSA increments on absorbance calculated by densitometry was used to predict correlation (Fig. 1). A significance level of 0.05 was set for statistical tests.

Results

As expected in such a series, all children responded to raw beef, cooked beef, and unheated BSA at SPT. Seven patients were sensitized by heated BSA, and none by HB, and only one reacted to FDB with a 7-mm-diameter wheal. None, including this same child, developed symptoms at HB or FDB challenge (Table 1). There was a positive correlation, and the test was statistically significant when the children were tested with BCE and unheated BSA and with BCE and heated BSA (Table 2). The correlations between a positive response to BCE and to both raw and cooked beef were less strong, and the tests did not reach statistical significance. Although heating BSA decreased wheal diameters in 8/10 children, two boys with a history of allergy (nos. 1 and 7, Table

1) had larger wheals with the heated extract. When unheated BSA was compared to either raw or cooked beef, Spearman's rho was 0.397 and this did not reach statistical significance. Heating BSA affected the correlation with raw beef downward but reached statistical significance ($P=0.016$) and more strongly correlated with beef only when it was cooked. The threefold increase in the magnitude of the correlation when heated BSA was compared with cooked beef emphasizes the altered reactivity of beef brought about by heating. Raw and cooked beef elicited wheal diameters that exhibited a moderate degree of correlation, while unheated and heated BSA both significantly and strongly correlated. The DBPCFCs were negative for both HB and FDB. An immediate-onset reaction to unheated BSA developed in five children, four of whom were also sensitized to heated BSA. However, after blinded challenges, systemic response did not markedly differ between heated and unheated BSA, and this limited series did not allow symptom severity assessment. The child (no. 3, Table 1) who first developed lip edema did not do so at recall challenge.

Discussion

BCE is not standardized and its allergen composition is unknown. However, as an unheated extract, it should contain most of the proteins present in beef, BSA, actin, and tropomyosin featuring among the allergenic fractions. BCE is defatted in solvents, but protein matrices may pass intact into the preparation. From the high level of significance reached when BCE-induced reactions were compared with wheals induced by heated and

Table 1. Patient characteristics and results of SPT and DBPCFC

Patients	1	2	3	4	5	6	7	8	9	10
Age (years)	2.25	1.58	4.33	3.00	1.25	1.33	2.50	3.66	2.83	4.41
Sex	M	F	M	F	M	M	M	F	M	F
Wheal diameter in mm										
BCE	12.5	12.5	28.2	3.1	7.0	3.1	28.2	7.0	12.5	12.5
Raw beef	19.6	12.5	12.5	7.0	7.0	7.0	12.5	19.6	12.5	19.6
Cooked beef	12.5	3.1	7.0	3.1	12.5	3.1	12.5	7.0	7	7.0
HB	—	—	—	—	—	—	—	—	—	—
FDB	—	—	7.0	—	—	—	—	—	—	—
Unheated BSA	12.5	12.5	38.5	12.5	12.5	7.0	12.5	12.5	38.5	12.5
Heated BSA	28.2	—	28.2	3.1	7.0	—	28.2	—	12.5	3.1
Results of DBPCFC										
HB	—	—	—	—	—	—	—	—	—	—
FDB	—	—	—	—	—	—	—	—	—	—
Unheated BSA	L	—	L	—	—	—	R, A	—	U	U
Heated BSA	L	—	—	—	—	—	R, C	—	L	U

A: asthma; C: cough; L: lip edema; R: rhinitis; U: urticaria; BSA: bovine serum albumin; BCE: beef commercial extract; HB: homogenized beef; FDB: freeze-dried beef.

unheated BSA, we can assume that such a composition imbalance increases the allergenic potency of BCE. The capability of BCE and extracted BSA as allergens for priming skin and systemic reactions for diagnostic purposes cannot be quantified because these preparations are not standardized, but neither can it be invalidated in this series (no false negatives). The strong, positive correlation between heated and unheated BSA-induced wheals also shows the stability and resistance to heat of the allergenicity of this protein. From the relatively low level of correlation between BCE and both raw and cooked beef on the one hand and between raw and cooked beef on the other, we infer that the cooking temperatures in this trial could not deactivate beef allergens. An IgE-immunoblot study (7) has found that proteins still showing in the 66-kDa band after 5-min cooking at 80°C did not bind after heating for 20 min at 84.5°C. This supports the case for longer cooking times at higher temperatures to break up the protein matrix of BSA. Only immunoblots of patient sera could map the progressive structural alterations of BSA at various temperatures. We thus argue that industrial preparations may be safer because of the high-temperature, pressurizing and sterilizing cycles which proteins undergo during processing. Taken together, our data suggest that home cooking does not rule out exposure to active allergens and that the temperature range adopted for this trial cannot protect patients from BSA-induced allergic symptoms.

HB and FDB have been shown not to evoke allergic sensitization at SPT among beef-allergic

Table 2. Results of Spearman's rank correlation test (r) between wheal diameters at SPT ($P=0.05$)

Allergens	r	P
BCE vs raw beef	0.518	0.123
BCE vs cooked beef	0.536	0.104
BCE vs unheated BSA	0.678	0.031
BCE vs heated BSA	0.703	0.024
Unheated BSA vs raw beef	0.397	0.247
Unheated BSA vs cooked beef	0.397	0.247
Heated BSA vs raw beef	0.209	0.537
Heated BSA vs cooked beef	0.739	0.016
Unheated BSA vs heated BSA	0.642	0.044
Raw beef vs cooked beef	0.518	0.123

BSA: bovine serum albumin; BCE: beef commercial extract.

children (9). From a nutritional point of view, the fact that neither HB nor FDB evoked a clinical reaction during challenges is suggestive. If freeze-drying or repeated heat treatment can deactivate BSA and other bovine allergens, useful proteins from selected cattle breeds may be allowed into the diet of children with beef intolerance who may also suffer from cow's milk allergy as well as other food allergies (15–19). However, clinical reactivity to processed meats has been reported in one study (7), and the possibility that an allergenic fraction remains in industrial products cannot be excluded. Sufficient information on cooking times and temperatures should be collected from manufacturers, and a DBPCFC should be planned before considering dietary introduction of beef products in such cases.

Acknowledgment

We acknowledge Gabriel R. Bouygue's contribution to the paper.

References

1. Yunginger SJW. Food antigens. In: Metcalfe DD, Sampson HA, Simon RA, editors. Food allergy: adverse reactions to foods and food additives. 2nd ed. Cambridge, MA: Blackwell Science, 1997:49-63.
2. Sampson HA. The role of food allergy and mediator release in atopic dermatitis. *J Allergy Clin Immunol* 1988; 81:635-45.
3. Sampson HA. Role of immediate hypersensitivity in the pathogenesis of atopic dermatitis. *Allergy* 1989;44:52-8.
4. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985; 107:669-75.
5. Sampson HA. Food hypersensitivity as a pathogenetic factor in atopic dermatitis. *Allergy Proc* 1986;7:511-19.
6. Fiocchi A, Restani P, Riva E, et al. Meat allergy. I. Specific IgE to BSA and OSA in atopic, beef-sensitive children. *J Am Coll Nutr* 1995;14:239-44.
7. Werfel SJ, Cooke SK, Sampson HA. Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 1997;99:293-300.
8. Wahn U, Peters T, Siraganian RP. Allergenic and antigenic properties of bovine serum albumin. *Mol Immunol* 1981; 18:19-28.
9. Fiocchi A, Restani P, Riva E, et al. Meat allergy. II. Effects of food processing and enzymatic digestion on the allergenicity of bovine and ovine meats. *J Am Coll Nutr* 1995;14:245-50.
10. Restani P, Fiocchi A, Restelli AR, et al. Effect of technological treatments on digestibility of meat-based baby foods. *J Am Coll Nutr* 1997;16:376-82.
11. Ortolani C, Ispano M, Pastorello ED, Ansaloni R, Margi GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989; 83:683-90.
12. Restani P, Restelli AR, Capuano A, Galli CL. Digestibility of technologically treated lamb meat samples evaluated by an *in vitro* multienzymatic method. *J Agr Food Chem* 1992; 40:989-93.
13. Bock SA, Sampson HA, Atkins FM. DBPCFC as an office procedure: a manual. *J Allergy Clin Immunol* 1988;82: 986-92.
14. Bock SA. *In vivo* diagnosis: skin testing and oral challenge procedures. In: Metcalfe DD, Sampson HA, Simon RA. Food allergy: adverse reactions to foods and food additives. 2nd ed. Cambridge, MA: Blackwell Science, 1997:161.
15. Giovannini M, Fiocchi A, Agostoni C, Riva E. Nutrition in infancy and childhood. In: Wüthrich B, Ortolani C, editors. Highlights in food allergy. Monogr Allergy 1996; 32:25-9.
16. Restani P, Fiocchi A, Beretta B, Velonà T, Giovannini M, Galli CL. Meat allergy: proteins involved and cross-reactivity between different animal species. *J Am Coll Nutr* 1997;16:383-9.
17. Speer F. Multiple food allergy. *Ann Allergy* 1975;34:71-6.
18. Metcalfe DD. Food hypersensitivity. *J Allergy Clin Immunol* 1983;73:749-61.
19. Vouillot C, De Blay F, Thierry R, Simon B, Mahr L, Pauli G. Chicken albumin responsible for cross-sensitization between eggs and chicken liver and flesh. *J Allergy Clin Immunol* 1992;89:194-200.