

SHORT REPORT

Report of occupational asthma due to phytase and β -glucanase

T M O'Connor, J F Bourke, M Jones, N Brennan

Abstract

Objectives—Occupational asthma is the principal cause of respiratory disease in the workplace. The enzymes phytase and β -glucanase are used in the agricultural industry to optimise the nutritional value of animal feeds. A relation between these enzymes and occupational asthma in a 43 year old man was suspected.

Methods—Inhalation challenge tests were performed with the enzymes phytase, β -glucanase, and amylase. Skin prick tests were performed with the enzymes diluted to a concentration of 1 mg/ml and 5 mg/ml. Specific IgE to phytase and β -glucanase were measured with a radioallergosorbent test.

Results—Baseline spirometry values were normal. A histamine challenge test showed bronchial hyperreactivity. Exposure to phytase and β -glucanase led to significant reductions in forced vital capacity and forced expired volume in 1 second. No significant differences were noted after exposure to amylase. Skin tests showed a positive reaction to β -glucanase (5 mm) at a concentration of 1 mg/ml and positive reactions to β -glucanase (7 mm) and phytase (5 mm) at a concentration of 5 mg/ml. Similarly specific IgE was present against both phytase and β -glucanase, at 2.5% and 9.3% binding respectively (2% binding is considered positive).

Conclusions—This is the first description of occupational asthma due to the enzymes phytase and β -glucanase. Their addition to the ever increasing list of substances associated with occupational asthma will have notable implications for those exposed to these enzymes.

(*Occup Environ Med* 2001;58:417–419)

Keywords: occupational asthma; phytase; β -glucanase

Occupational asthma may account for 4%–10% of all cases of asthma and is the principal cause of respiratory disease in the workplace environment.¹ It is defined as asthma causally and specifically related to exposure to airborne dusts, gases, vapours or fumes in the working

environment, and many patients have continued asthma despite ending exposure. More than two thirds of cases are attributed to sensitisation.²

Phytase is an enzyme that is secreted by several species of yeast, and is used in the agricultural industry to catalyse the hydrolysis of phytic phosphorous in animal feeds.³ This leads to increased inorganic phosphate absorption, giving rise to improved growth and bone mineralisation, as well as reducing the amount of phosphate excreted by the animal into the environment. β -Glucanase reduces intestinal viscosity when added to animal feeds and increases the activity of other enzymes—such as amylase, lipase, and trypsin—leading to improved weight gain and nutrition.⁴ Cellulase and amylase have similar positive effects on growth.^{5,6}

Based on clinical history, we suspected a relation between the enzymes phytase and β -glucanase and occupational asthma in a patient.

Methods

A 43 year old company director of an animal feed manufacturing plant was referred by his general practitioner. He described an 8 week history of wheezing and coughing that began on arrival at work and abated while away from the factory. Asthma had been diagnosed 7 years previously by his general practitioner, and had been well controlled on inhaled steroid and β -agonist therapy. There was no history of allergic rhinitis or contact dermatitis. He was a non-smoker. There was a strong family history of asthma (mother, brother, and son were affected). He had been employed in his current occupation for 14 years. None of 22 other workers in the factory had asthma or had developed respiratory symptoms at work. His company had begun to manufacture animal feeds containing the enzyme phytase 1 year previously. The main other components of these feeds were various vitamins, trace elements, and the enzymes amylase and β -glucanase. β -Glucanase had been introduced to the factory 9 years previously.

Physical examination indicated expiratory wheeze. A chest radiograph was normal and serum IgE was 48 KU/l (normal <100 KU/l). Histamine challenge tests were performed as previously described.⁷ Inhalation challenge

Department of
Respiratory Medicine,
Mercy Hospital, Cork,
Ireland

T M O'Connor
N Brennan

Department of
Dermatology, South
Infirmiary Victoria
Hospital, Cork, Ireland
J F Bourke

Department of
Occupational and
Environmental
Medicine, Imperial
College, London, UK
M Jones

Correspondence to:
Dr T M O'Connor,
Department of Respiratory
Medicine, Cork University
Hospital, Wilton, Cork,
Republic of Ireland
terryoconnor@eircom.net

Accepted 9 February 2001

Table 1 Spirometric values (l) before and after exposure to enzymes

Enzyme	Spirometry	Baseline	After challenge	% Change
Amylase	FVC	4.50 (93)	4.53 (93)	0
	FEV ₁	3.17 (80)	3.08 (78)	-3
β -glucanase	FVC	4.70 (97)	3.85 (79)	-18
	FEV ₁	3.41 (86)	2.53 (64)	-26
Phytase	FVC	4.54 (94)	4.02 (83)	-11
	FEV ₁	3.92 (99)	2.82 (71)	-28

Predicted FVC 4.85l, predicted FEV₁ 3.96l, percentage of predicted values in parentheses.

tests were performed with the enzymes phytase, β -glucanase, and amylase at weekly intervals, the patient having avoided the workplace for 10 days before testing. The patient was asymptomatic at the beginning of each challenge, and minor variations found in baseline spirometry values were thought to reflect the natural variability in these values found in patients with asthma. Pure enzymes were obtained in powder form from their manufacturers, and the patient was exposed by pouring the powder from cup to cup near his face for 3 minutes. Pulmonary function was performed with the Jaeger Masterlab spirometer. The same technician performed all the tests. The tests were performed with the subject sitting in a chair breathing through a mouthpiece with a nose clip. The best results of three flow volume manoeuvres were used. Spirometry values were obtained immediately before and 15 minutes after exposure. Pulmonary function was performed in accordance with the guidelines recommended by the American Thoracic Society.⁸

Skin prick tests were performed with positive and negative controls and with the enzymes diluted to a concentration of 1 mg/ml and 5 mg/ml. Reactions were measured at 15 minutes. Reaction of 5 mm or more, and 3 mm more than the negative control were considered positive. Specific IgE to phytase and β -glucanase were measured with radioallergen sorbent test. Briefly, 3 mg of allergen was coupled to 300 mg cyanogen activated paper discs according to the method of Ceska *et al.*⁹ For the assay, serum (50 μ l) was added to a disc. After incubation at room temperature for 16 hours, the disc was washed and added to 50 μ l ¹²⁵I-antihuman IgE. After 16 hours at room temperature, the disc was washed and counted in a γ scintillation counter. The amount of antigen specific IgE was expressed as the percentage of the added counts per minute (cpm) that remained bound to the disc (percentage binding). Two per cent binding is chosen conventionally as a positive cut off; 1%–2% is considered to be borderline. Unexposed populations do not exceed the 1% RAST binding level. Cord blood was used as a negative control.

Results

Histamine challenge testing showed bronchial hyperresponsiveness (provocative concentration causing a 20% fall in forced expired volume in 1 second (PC₂₀) was 1 mg/ml). Skin tests showed a positive reaction to β -glucanase (5 mm) at a concentration of 1 mg/ml and

positive reactions to β -glucanase (7 mm) and phytase (5 mm) at a concentration of 5 mg/ml. There were no significant reactions to amylase. Similarly specific IgE was present against both phytase and β -glucanase, with 2.5% and 9.3% binding respectively. Cord blood was negative in both RAST assays.

Baseline spirometry values were normal. On exposure to the enzymes phytase and β -glucanase, the patient developed symptoms of cough, wheeze, and rhinorrhoea. Spirometry values after exposure showed significant reductions in forced vital capacity and forced expired volume in 1 second (table 1). No significant differences were noted after exposure to amylase.

Discussion

Occupational asthma has been described in association with the enzymes amylase and cellulase.^{10,11} Although there is evidence of occupational IgE sensitisation to phytase, there is no known direct association between occupational asthma and phytase or β -glucanase to date.¹² The exact extent of industrial exposure to these enzymes is unknown. β -Glucanase has been in widespread use since the 1960s but phytase has been increasingly used as an animal feed additive since the early 1990s.¹² Thus, occupational asthma resulting from exposure to these enzymes may be an unrecognised problem in the animal feed manufacturing industry. This is the first report of the direct association of occupational asthma with the enzymes phytase and β -glucanase.

Occupational asthma is a common condition, which is steadily emerging as the principal cause of respiratory disease in the workplace and may be caused by several hundred agents.¹ Pre-existing asthma does not prevent the diagnosis of subsequent occupational asthma in a person. The level of exposure is an important risk factor for the development of occupational asthma, and reduction or avoidance of exposure is the only certain method of prevention. Once asthma develops, many patients experience continued symptoms despite ending exposure. Early diagnosis and early removal from exposure are the most important factors for improving the long term outcome.

Phytase and β -glucanase are secreted by numerous fungal species. Phytase is predominantly derived from *Aspergillus niger* and β -glucanase from *Aspergillus* and *Bacillus* species in the animal feed manufacturing industry. Phytase may cross react with other *Aspergillus niger* mould extracts.¹² Phosphorous is an essential component of many organic and inorganic compounds in vertebrates and therefore, an adequate dietary phosphorous supply is important. In feedstuffs of plant origin, most of the phosphorous is bound within phytate (myoinositol hexakisphosphate), and can only be absorbed after enzymatic breakdown of phytic acid by phytases, which catalyse the hydrolysis of phytate to myoinositol and inorganic phosphate.³ Phytic acid decreases the bioavailability of many essential minerals by interacting with multivalent cations or proteins to form complexes that may be insoluble or otherwise

unavailable under physiological conditions.¹³ Therefore, supplementation of animal feeds with phytase improves absorption of inorganic phosphate, essential minerals, and trace elements, improves consequent growth, nutrition, and bone mineralisation, and reduces phosphorous excretion into the environment.

Barley based diets in animals contain mixed link β -glucans, which increase viscosity in the small intestine. The addition of β -glucanase to animal feeds leads to hydrolysis of β -glucans, reducing viscosity, increasing the activity of other enzymes and the digestibility of organic matter, and consequently improving growth.⁴

The addition of phytase and β -glucanase to the ever increasing list of substances linked with occupational asthma will have significant implications for those exposed to these enzymes. Because occupational asthma may cause long lasting disability, it is important to identify affected workers correctly and to remove them as soon as possible from the sensitising agent as well as protecting asymptomatic workers by controlling inhalational exposures to these agents.

1 Kogevinas M, Anto JM, Sunyer J, *et al.* Occupational asthma in Europe and other industrialised areas: a population-based study. European Community Respiratory Health Survey Study Group. *Lancet* 1999;353:1750-4.

- 2 Keynes HL, Ross DJ, McDonald JC. SWORD '95: surveillance of work-related and occupational respiratory disease in the UK. *Occup Med (Lond)* 1996;46:379-81.
- 3 Mitchell DB, Vogel K, Weimann BJ, *et al.* The phytase sub-family of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*. *Microbiology* 1997;143:245-52.
- 4 Almirall M, Francesch M, Perez-Vendrell AM, *et al.* The differences in intestinal viscosity produced by barley and β -glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *J Nutr* 1995;125:947-55.
- 5 Feng P, Hunt CW, Pritchard GT, *et al.* Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. *J Anim Sci* 1996;74:1349-57.
- 6 Ritz CW, Hulet RM, Self BB, *et al.* Growth and intestinal morphology of male turkeys as influenced by dietary supplementation of amylase and xylanase. *Poult Sci* 1995;74:1329-34.
- 7 Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760-5.
- 8 Gardner RM, Hankinson JL, Clansen JL, *et al.* Standardisation of spirometry: 1987 update. Official statement of the American Thoracic Society. *Am Rev Respir Dis* 1987;136:1285-98.
- 9 Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1-9.
- 10 Smith TA, Smith PW. Respiratory symptoms and sensitization in bread and cake bakers. *Occup Med (Lond)* 1998;48:321-8.
- 11 Losada E, Hinojosa M, Moneo I, *et al.* Occupational asthma caused by cellulase. *J Allergy Clin Immunol* 1986;77:635-9.
- 12 Doekes G, Kamminga N, Helweggen L, *et al.* Occupational IgE sensitisation to phytase, a phosphatase derived from *Aspergillus niger*. *Occup Environ Med* 1999;56:454-9.
- 13 Cheryan M. Phytic acid interactions in food systems. *Crit Rev Food Sci Nutr* 1980;13:297-335.

Correspondence and editorials

Occupational and Environmental Medicine welcomes correspondence relating to any of the material appearing in the journal. Results from preliminary or small scale studies may also be published in the correspondence column if this seems appropriate. Letters should be not more than 500 words in length and contain a minimum of references. Tables and figures should be kept to an absolute

minimum. Letters are accepted on the understanding that they are subject to editorial revision and shortening.

The journal also publishes editorials which are normally specially commissioned. The Editor welcomes suggestions regarding suitable topics; those wishing to submit an editorial, however, should do so only after discussion with the Editor.