

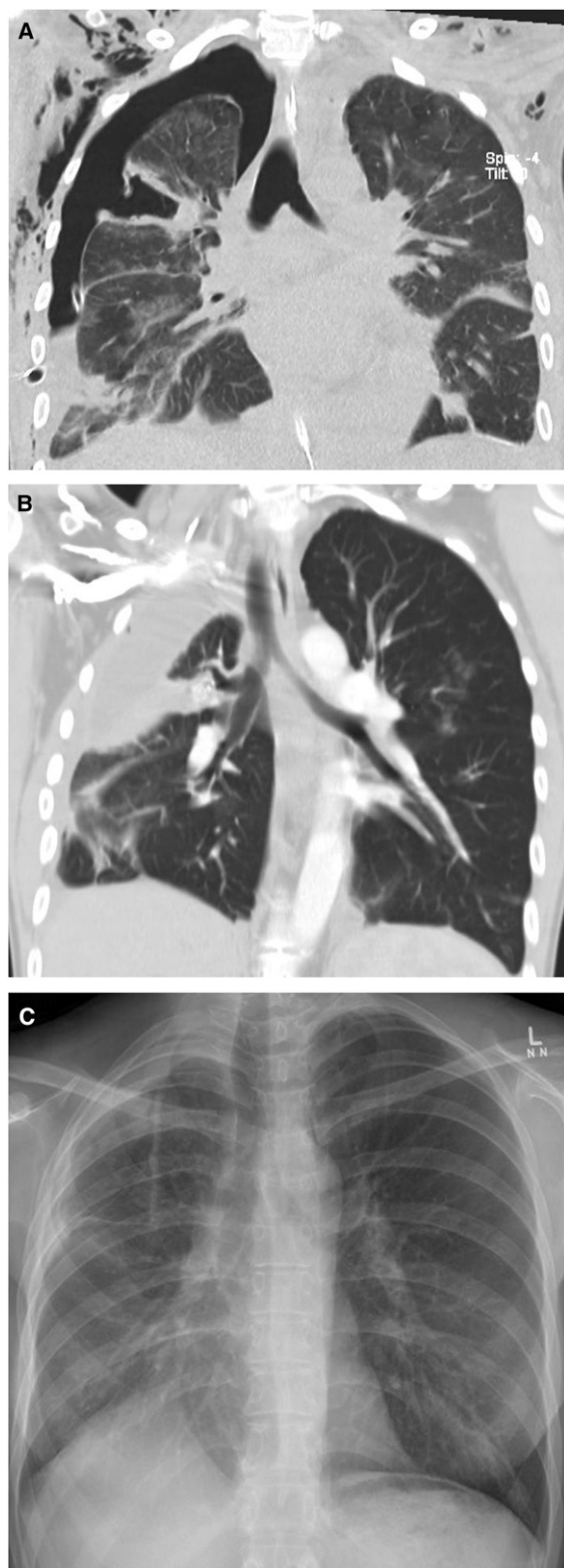


Figure 1. (A) Computed tomography chest (coronal view) at the diagnosis of bronchopleural fistula showing large right upper lobe (RUL) cavity communicating with pleura. The first ICC is *in situ* lower right. (B) Computed tomography chest (coronal view) after placement of three endobronchial valves (EBVs) in RUL. The valves can be seen at origin of RUL. The RUL is completely collapsed. A residual space remains outside this due to inability of right middle and lower lobe to expand due to coexistent emphysema. (C) Chest radiograph at 6 months showing only minor R lung volume loss, small amount of residual atelectasis in RUL and minor pleural changes.

the treatment of choice (10, 11). The case raises the question whether EBV treatment might also be used electively in patients who have pulmonary cavities with chronic complications such as significant hemoptysis, particularly in poor operative candidates.

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Protein Identification of Two Allergens of *Boletus edulis* Causing Occupational Asthma

To the Editor:

Occupational respiratory diseases associated with exposure to mushroom spores have been reported in recent years (1). *Boletus edulis*, a highly valued edible mushroom, has been implicated in both ingestive (2–4) and inhalative (2, 5, 6) allergy. Although *B. edulis* allergens have occasionally been characterized using immunoblotting, to our knowledge their proteins have not been identified to date. We report a patient who developed

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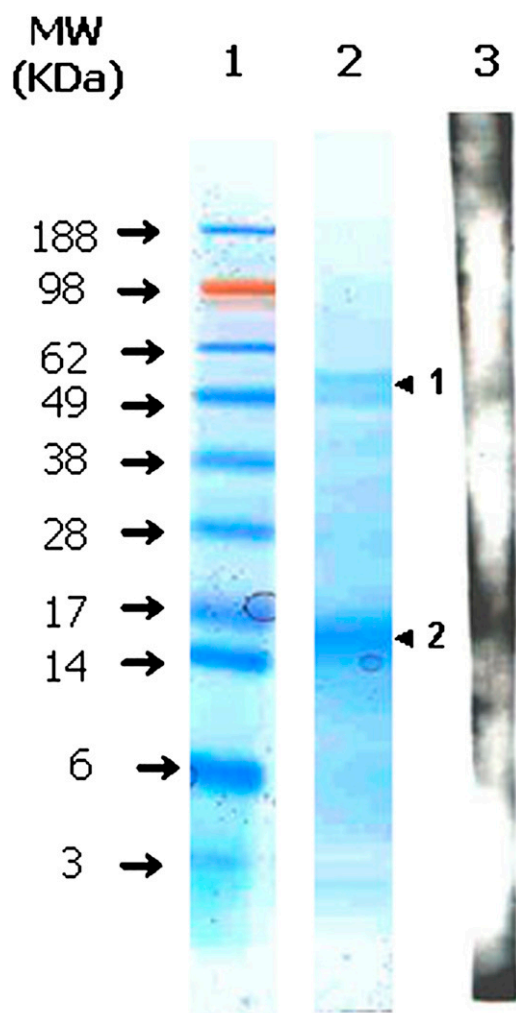


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting results. (Lane 1) Molecular weight markers. (Lane 2) Coomassie-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis of *Boletus edulis* extract. (Lane 3) Immunoblotting of patient serum (IgE demonstrated intense binding to the *B. edulis* proteins).

occupational asthma after exposure to *B. edulis* dust, in which protein identification was possible thanks to DeNovo sequencing of the proteins responsible for respiratory allergy.

The patient was a nonatopic, nonsmoking 58-year-old woman, who had been working as a kitchen assistant in a restaurant for the last 6 years. Six months after starting work, she had experienced dyspnea, coughing, and wheezing every time she handled *B. edulis*, and had consulted the hospital emergency service on three

occasions for episodes of severe bronchospasm. The patient manipulated only the edible part of *B. edulis*, which was purchased in dried form in commercial preparations of 200 g.

The blood test revealed 7,200 leukocytes with 4% of eosinophils and a total IgE of 134 U/ml. Chest radiography was normal. Skin prick test was positive to a patient *B. edulis* extract (10% w/v). Prick tests to common pneumoallergens were normal. Spirometry revealed FVC and FEV₁ of 88% and 77% of the theoretical value, respectively, with a ratio of 65%. The methacholine inhalation test showed a provocative concentration of methacholine causing a 20% fall in FEV₁ of 0.25 mg/ml. A specific inhalation challenge was performed by tipping 10 g of *B. edulis* mixed in 150 g of lactose from one tray to another at a distance of 30 cm from the nose, as previously described (7). After 7 minutes of exposure, the patient presented dyspnea, coughing, and wheezing with a fall in FEV₁ of 49% compared with baseline, which recovered after administration of a β_2 agonist. This response was not observed after inhalation of placebo (lactose) the day before the test.

Immunoblotting performed with the *B. edulis* power extract showed two IgE-binding bands at approximately 17 and 55 kD (Figure 1). Protein identification was performed with DeNovo sequencing using PEAKS Studio 5.3 software (Bioinformatics Solutions Inc., Waterloo, ON, Canada). Briefly, the *B. edulis* protein bands separated by one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis were excised from the gel and digested with trypsin according to standard protocols. The tryptic peptides were analyzed with a maXis UHR-Qq-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) and subsequently sequenced using both the DeNovo and the Spider Homology Search tools from the PEAKS Studio 5.3 software. The mass spectrometry identified a 16 kD band as a fungal fruit body lectin and a 46 kD band as a protein of the glycoside hydrolase family (Table 1).

The sequencing of the proteins responsible for respiratory allergy to *B. edulis* is the main contribution of this study. The first two cases of asthma due to *B. edulis* were described by Torricelli and colleagues (2) in 1997. In 2005, Baruffini and coworkers (5) described a new case in a female working in the selection and packing of mushrooms. More recently, in 2008, Foti and colleagues (6) suggested that components at approximately 17, 55, and 88 kD may be responsible for IgE-mediated respiratory allergy to *B. edulis*. Our case confirms that the proteins implicated in the inhalatory allergy to this mushroom are most likely the ones at 16 and/or 46 kD. However, the proteins reported to cause food allergy may differ from the ones described here, as there are discrepancies among the few existing studies. In this regard, Roncarolo and colleagues (3) described two thermostable IgE-binding bands at 26 and 39 kD in the serum of a patient with allergic symptoms after ingestion of fresh raw or cooked dried *B. edulis*. Helbling and colleagues (4) studied two subjects, one with recurrent anaphylaxis and the other with a predominantly oral allergy syndrome following

TABLE 1. IDENTIFICATION OF *Boletus edulis* IgE-BINDING BANDS BY DeNovo SEQUENCING

Band ID*	MW (kD) [†]	Protein Identified (Theoretical MW in kD) [‡]	Accession Number (GI) [§]	Sequence Coverage (%)	Sequence
1	50	Glycoside hydrolase family 5 protein (46.35)	336364739	19	IGYWAFEVGPGE/PYIQGQLPYLQKAVTWAGNHGLK/IAPLNEPAGFDGAAVLNAT/QFWEAQVISYEK/AENADDWSYEAGLA
2	16	Fungal fruit body lectin (16.01)	75765497	24	ANGAHTLTQGGSGTSGVLR/EKQLAEYSVTS/AIGTK

Definition of abbreviation: MW = molecular weight.

*Each band is described by its number on the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Figure 1).

[†]Apparent MW extrapolated from the calibration curve.

[‡]Proteins were identified by DeNovo sequencing.

[§]Accession number in the National Center for Biotechnology Information database.

^{||}Percentage of the protein covered by matched peptides.

ingestion of *B. edulis*, and described three proteins, the most reactive ones at 55 and 80 kD, and the other at 75 kD, but only in the sera of the subject with recurrent anaphylaxis.

Although few cases of occupational asthma due to inhalation of *B. edulis* have been described to date (2, 5, 6), its prevalence may well be increasing due to its wide use in the food industry. The sequencing of the proteins responsible may be a first step forward in the design of future therapeutic strategies.

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Effects of Acute Intermittent Hypoxia on Working Memory in Young Healthy Adults

Obstructive sleep apnea (OSA) is characterized by chronic intermittent hypoxia (IH) during sleep and is frequently accompanied by impairment in several cognitive domains, including working memory (i.e., the processes used to temporarily maintain and manipulate information in mind) and executive control (1–6). A variety of factors are thought to contribute to working memory and executive deficits in patients with OSA, including IH, sleep fragmentation, daytime sleepiness, and comorbid medical disorders (e.g., obesity and high blood pressure) (7–9). However, the contribution of IH to the development of working memory deficits, independent of other changes and comorbidities that accompany sleep apnea, is unknown and difficult to distinguish

in this patient population. We examined the hypothesis that selective exposure to acute IH can directly result in decreased performance on working memory tasks, using healthy adults to eliminate the confound of typical comorbidities.

Seventeen healthy young male volunteers were included in two sequential studies (n = 8 for study 1 and n = 9 for study 2) that were approved by the Conjoint Health Research Ethics Board. In both studies, participants were excluded if they had a prior diagnosis or history of cardiopulmonary or renal disease, sleep apnea, obesity, smoking within the past year, hypertension, or a high fasting blood glucose level. These criteria helped to confirm that all subjects were healthy and did not have medical conditions that could alter cardiorespiratory function (see online data supplement in Reference 10 for a detailed description of the inclusion/exclusion criteria, screening sessions, and baseline measurements).

Study 1 reports unpublished data on the assessment of working memory function during two conditions from Foster and colleagues' 2010 study (10) including 8 of the original 10 participants. Working memory was assessed during the placebo + IH and sham IH conditions in Foster and colleagues' 2010 study (10), giving us an opportunity to examine the effects of IH compared with normoxia, respectively. The third condition (losartan + IH) was not included in the working memory assessment (see Reference 10 for a detailed description of the three conditions). Two of the original ten participants were eliminated from study 1 due to noncompliance and previous exposure to losartan. The two conditions of interest in the present report will be referred to as experimental IH and sham IH (normoxia) conditions, respectively. Study 1 involved an experimental randomized crossover design in which participants (mean [SD] age = 30 [0.7] yr) were exposed to 6 hours of IH (experimental condition) or normoxia (sham condition) in a normobaric chamber on 2 separate days (see Figure 1A). Study 2 was conducted after study 1 to investigate the possibility of a practice effect on n-back performance over the two testing sessions in study 1. This study was not reported in Foster and colleagues' 2010 article (10) as it focused exclusively on the n-back behavioral performance. In study 2, 10 participants (mean [SD] age = 30 [2.9] yr) were exposed to 6 hours of normoxia on two separate days (see Figure 1B), but one participant was eliminated from analyses based on poor performance (reaction times [RTs] were 2–3 SD outside the group average). In both studies, each testing session was separated by a minimum of 1 week (mean [SD] duration = 13 [5.9] d) and occurred at the same time of day. Exposure to IH consisted of

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