Bronchial provocation testing with cold air was carried out on 36 asthmatic and 13 normal subjects in order to assess the reproducibility and clinical relevance of the technique as a test of airways reactivity. Sixteen subjects underwent repeat testing after an interval of two to three weeks. Using a least squares linear regression analysis, the technique was highly reproducible, with a correlation of \( r = 0.93 \) \( (p < 0.001) \). The 21 asthmatic subjects who had exercise-provoked symptoms required a significantly lower level of ventilation of cold air to produce a 35 percent drop in specific airways conductance \( (PD_{35}) \) than did those who had no exercise-induced asthma \( (33.9 \text{ L min}^{-1} \text{ vs } 45.8 \text{ L min}^{-1}; p < 0.02) \). Subjects requiring no regular treatment for their asthma had a geometric mean \( PD_{35} \) of 62.6 L min\(^{-1} \), significantly higher than those requiring inhaled therapy \( (44.9 \text{ L min}^{-1}; p < 0.005) \). Subjects requiring oral in addition to inhaled treatment had the lowest \( PD_{35} \) \( (23.6 \text{ L min}^{-1}; p < 0.02) \). Atopic status did not appear to influence the response. There was a strong correlation between the \( PD_{35} \) to cold air and to histamine \( (r = 0.92; p < 0.001) \) and between the \( PD_{35} \) to cold air and to methacholine \( (r = 0.86; p < 0.001) \). The three techniques of assessing bronchial reactivity were equally successful in separating the normal and asthmatic groups. The results indicate that cold air provocation may be reliably and reproducibly used to assess bronchial reactivity. The use of a naturally-occurring stimulus of asthma in all subjects has great potential as an investigative technique.

The observation that the inhalation of cold air could provoke an attack of asthma in susceptible subjects has been recognized for several hundred years. More recent studies confirmed that bronchoconstriction could be provoked in subjects with obstructive airways disease by the inhalation of air at subfreezing temperatures, and Simonssen et al showed that asthmatic subjects were "hyperreactive" to the stimulus, just as they were to inhaled histamine. With the recognition of the interrelationship between airway cooling and exercise-induced asthma, increasing attention has been focused on cold air as a technique for assessing bronchial reactivity, and equipment to enable pulmonary function laboratories to perform cold air provocation testing is now commercially available.

In this paper, the clinical relevance of cold air as a provocation technique is evaluated by assessing its reproducibility and comparing it with histamine and methacholine challenges in asthmatic and nonasthmatic subjects. We also document the variability in sensitivity to cold air between asthmatic subjects with differing severity of asthma as indicated by their minimum treatment requirements.

**METHODS**

**Subjects**

A total of 49 subjects, 36 suffering from bronchial asthma and 13 normal subjects, were studied. Anthropometric details are shown in Table 1. Normal subjects had no recent or remote history of lung disease, had baseline lung function measurements within their normal predicted range, and had been free of upper respiratory infection for at least six weeks prior to any study. Asthmatic subjects all had documented reversibility of airflow obstruction of at least 15 percent. Treatment requirements ranged from no regular treatment to oral corticosteroids and theophylline, in addition to regular inhalation therapy. Prior to the provocation tests, the asthmatic patients were in a stable clinical state and their treatment regimens were the minimum necessary to give satisfactory control of their symptoms, ie, they had no sleep disturbance, were free of wheeze on

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<th>Table 1—Patient Summary</th>
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Cold Air as Bronchial Provocation Technique (Heaton, Henderson, Costello)
waking in the morning, and were able to carry out their normal daily routines without being limited by their asthma.

Inhaled beta-agonists and disodium cromoglycate were discontinued for at least 12 hours prior to any study and oral theophylline preparations for 24 hours. Baseline lung function tests varied by less than 10 percent in any subject on any study day. For patient safety, no subject was studied whose resting FEV₁ on the day of the test was less than 65 percent of his/her predicted normal value; for this reason, severely asthmatic patients were not studied.

All subjects gave their informed consent to the studies.

Cold Air Challenge

The apparatus and technique used for delivering a cold air challenge are described in detail elsewhere.¹⁹ Dry air is passed across the cooling coil of a Haake EH31 refrigeration unit inside an insulated cooling chamber. A valve system separates the inspired and expired air. The subject's level of ventilation is set by requiring him to keep inflated a prefilled target balloon which is evacuated at the required rate by a variable vacuum pump. Inspired and expired air temperatures are recorded. End-tidal CO₂ concentration is also measured and CO₂ fed into the inspired air to maintain eucapnia. Responses to the challenge are measured in terms of changes in specific airways conductance (Gaw/Vl), measured in a constant volume, whole body plethysmograph. Five measurements of Gaw/Vl air were made at each time interval and the mean value taken.

Two techniques were used for assessing an individual's response to the provocation test. To assess the reproducibility of the test, the response to a single level of ventilation was compared on two occasions separated by an interval of two to three weeks. Asthmatic subjects were required to breathe cold air for three minutes at a minute ventilation of 15 times their baseline FEV₁, whereas the normal subjects were required to breathe at 30 times resting FEV₁. In the remaining studies, dose-response curves to cold air were established by repeating the challenge at 15 minute intervals with increasing minute ventilations from 10 to 30 times resting FEV₁, or until a 50 percent drop in Gaw/Vl had been achieved. From the log dose-response curves the "dose" of cold air (L min⁻¹) causing a 35 percent drop in Gaw/Vl was determined by interpolation or linear extrapolations of the last two points (PD₃₅).

Histamine-Methacholine Inhalation Tests

Inhalation tests were performed using a modification of the technique of Cockcroft et al.¹⁸ Solutions were administered for two minutes tidal inhalation through the open mouth using a Wright's nebulizer (output 0.13 ml/min; flow rate 7 L min⁻¹). After baseline determinations of Gaw/Vl, buffered saline solution was administered and then doubling concentrations of agonist from 0.0625 mg/ml to 16 mg/ml at five minute intervals. After each inhalation, Gaw/Vl was determined at 20-second intervals from one minute to three minutes postinhalation and the mean of these values taken as the response to that dose. Inhalations were stopped when the Gaw/Vl had fallen by 50 percent or after the maximum dose had been given. The PD₃₅ for histamine or methacholine was determined from the log dose-response curve as for cold air.

Statistical Analysis

Reproducibility of the cold air challenge was assessed by least-square linear regression of the responses to the two challenges. Logarithmic transformations of PD₃₅ were compared using Student's t-test for paired and unpaired data. Group data are expressed as the geometric means.

RESULTS

Reproducibility

Ten asthmatic and six normal subjects had two iden-

![Figure 1. Reproducibility of response to two cold air challenges separated by an interval of two to three weeks. There is a close-fit of the points around the line of identity (solid line).](http://journal.publications.chestnet.org/pdaccess.ashx?url=/data/journals/chest/21443/)

![Figure 2. PD₃₅ for cold air in asthmatic subjects with and without exercise induced asthma (EIA). Subjects with EIA have a significantly lower PD₃₅](http://journal.publications.chestnet.org/pdaccess.ashx?url=/data/journals/chest/21443/)
tical challenges in terms of minute ventilations and inspired air temperature separated by an interval of two or three weeks. The reproducibility is shown in Figure 1. There is a close grouping around the line of identity, with a correlation of $r = 0.93$ ($p < 0.001$).

**Influence of Symptoms and Treatment Requirements**

Twenty-one of the 36 asthmatic subjects gave a history of exercise-induced symptoms, whereas the remainder had never noticed wheezing during or following physical exertion. Figure 2 shows that although there is a considerable amount of overlap between the groups, the group with exercise-induced asthma had a significantly lower PD$_{35}$ for cold air than did those who had not experienced such symptoms (33.9 L min$^{-1}$ vs 45.8 L min$^{-1}$, $p < 0.02$).

Figure 3 shows the PD$_{35}$ for cold air in the asthmatic subjects divided into five treatment groups. Those subjects who required no regular therapy for their asthma had a significantly higher PD$_{35}$ than those requ

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21443/)
quiring inhalation therapy (62.6 L min⁻¹ vs 44.9 L min⁻¹ for those requiring an inhaled bronchodilator drug on a prn basis, p<0.005). There was no significant difference between the three groups of patients receiving inhaled therapy: 44.9 L min⁻¹ in those requiring bronchodilators, but not regularly; 38.2 L min⁻¹ in those on a regular daily bronchodilator regimen; and 39.7 L min⁻¹ in those taking beclomethasone dipropionate or disodium cromoglycate in addition to an inhaled bronchodilator. Subjects taking oral, in addition to inhaled, therapy had the lowest PD₉₀ (23.6 L min⁻¹; p<0.02) vs inhaled therapy. Atopic status did not appear to influence the PD₉₀ (Fig 3).

Comparison with Histamine and Methacholine Sensitivity

Thirty-two subjects (19 asthmatic and 13 normal) underwent both cold air and histamine provocation tests. Fifteen subjects (seven asthmatic and eight normal) were also challenged with methacholine. There was a strong correlation between the PD₉₀ to cold air and the PD₉₀ to histamine (r = 0.92; p<0.001); and between the response to cold air and to methacholine (r = 0.86; p<0.001).

Figure 4 compares the three techniques in separating normal and asthmatic subjects. As can be seen, in the subjects studied, all three techniques showed a clear separation of the groups, with no overlap between the range of PD₉₀ found in the two groups of subjects.

Discussion

Tests of airway reactivity have clinical, diagnostic and investigational relevance. Increased responsiveness to nonspecific stimuli is included in certain definitions of asthma and provocation testing may be carried out when the diagnosis of asthma is not clear cut. Occupational exposures may be associated with increased airways reactivity, and symptomatic asthma may occur in exposed workers. Lung function may decline at any increased rate in patients with chronic bronchitis who have hyperreactive airways. Studies into mechanisms of asthma or of the therapeutic potential of new drugs may require a method of safely inducing bronchospasm. In these studies, we have assessed the reproducibility and clinical relevance of testing airways reactivity using cold air as the provoking stimulus, and compared the results obtained using this challenge with more established inhalation challenge tests.

We have already reported the absence of a refractory period to cold air challenge and shown that the response is reproducible when the stimulus is repeated on the same day. This stability of response is maintained when the challenge is repeated after an interval of two to three weeks. Linear regression analysis shows a close-fit of the points about the line of identity (Fig 1), and the correlation coefficient of 0.93 compares favorably with the reported correlations for repeated histamine challenges.

The bronchoconstriction that occurs following exercise in asthmatic subjects appears to be closely related to airway cooling. Accordingly, it might be expected that those in whom exercise-induced asthma occurs would be more sensitive to airway cooling than those who do not have EIA; Figure 2 shows that this is the case. Although there is a significant difference between the mean of PD₉₀ in the two groups, there is a considerable amount of overlap. This might be explicable on the basis of individual differences in level of activity. However, it has been suggested that the asthmatic response to exercise is more complex than a simple dependence on respiratory heat exchange, and differing sensitivities to an “exercise stimulus” as opposed to a “cooling stimulus” may also be important.

For reasons of safety, no subject whose baseline FEV₁ was less than 65 percent of his/her predicted normal value was studied, and accordingly, more severely asthmatic patients were not studied. Only one subject was receiving regular treatment with oral corticosteroids. Within these limitations, there was a clear increase in bronchial reactivity with increasing treatment requirements. Similar results have been reported using histamine or exercise as the provoking stimulus. Again, there is overlap between the groups, but it is well recognized that factors other than airways reactivity, especially the degree of the immune response to specific allergen, influence the severity of clinical asthma.

Direct comparisons with the response to nonspecific pharmacologic stimuli of bronchoconstriction, histamine, and methacholine indicate that airways reactivity determined by any of these methods is comparable in normal and asthmatic subjects. This is in agreement with the results of O’Byrne et al who showed a close correlation between bronchial responsiveness to cold air and to methacholine. The three methods are also equally effective in separating the two groups of subjects.

These studies show that bronchial reactivity may be reliably and reproducibly measured in subjects with normal and hyperreactive airways using cold air provocation. The results obtained are comparable to those from histamine challenge tests. The advantage of using cold air rather than histamine is that bronchospasm is induced by complex mechanisms rather than by direct stimulation of airway smooth muscle. The use of a naturally-occurring stimulus of asthma in all subjects, including nonasthmatics, has great investigational potential. For reasons of patient safety and baseline stability, a call has recently been made for the increased use of normal subjects in asthma research,
especially in acute drug trials. Using cold air as a
stimulus to induce bronchoconstriction makes this a
real possibility, although it must be recognized that
mechanisms of response may differ in normal and asth-
matic subjects.13,15

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