Cold Air Bronchial Provocation

Technical Issues and Protocol

This document examines the underlying principles, technical issues and subtleties involved in the development of a generic protocol for the use of cold air isocapnic hyperventilation to aid in the diagnosis of asthma and related hyperreactive airway conditions. This information is intended as an educational tool to be used by medical personnel responsible for cold air challenge testing. Equilibrated Bio Systems, Inc. (EBS) does not assume any liability arising out of the application or use of any product described herein. Users shall indemnify and hold EBS and its officers, employees and distributors harmless against all such claims, costs, damages, expenses and attorney fees arising out of, directly or indirectly, any claim of personal injury or death associated with such use.

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I. Introduction

The purpose of this paper is to provide the reader with the guidelines and knowledge to develop a Cold Air Challenge (CACh) protocol. The underlying principles, technical issues and subtleties involved in the development of the protocol are provided as an educational tool to be used by medical personnel responsible for cold air challenge testing. The issues surrounding the theoretical basis of the protocol are thoroughly examined.

II. History of Cold Air Challenge

The CACh was originally developed to simulate the environment that triggers abnormal, acute airway obstruction associated with exercise.[1,2] This condition, referred to as Exercise Induced Asthma (EIA) or Exercised Induced Bronchospasm (EIB), is manifested by shortness of breath, coughing, wheezing and tightness in the chest shortly after the cessation of exercise.[3,4,28] Because EIB is often the only symptom found in patients suspected of having asthma, the ability to recreate the conditions (and quantify the airway limitation associated with it) takes on special significance in helping to make a diagnosis.

Initially, cold air was not used in the EIB testing procedure.[5] The test was done under ambient conditions and no one was certain of the exact mechanism responsible for the bronchoconstriction. Some believed it may have been due to the lactic acidosis or hypocapnia associated with exercise but, upon investigation, that was shown to be false.[3,4,6] After some time, however, a common denominator was discovered. It was found that the normal increase in ventilation that occurs during exercise (which cools and dries the inspired air) brought about an *abnormal* loss of heat and humidity in the airways of asthmatic patients -- this, in turn, triggered the bronchospasm.[1,7,8] To further substantiate the observation, it was demonstrated that if a patient inspired air (during exercise) that was heated to body temperature and fully saturated with water vapor, the bronchospasm could be prevented.[1,7,9] Conversely, it was also found that if dry sub frigid air was administered in conjunction with the EIB procedure, it hastened and intensified bronchospasm.[1,8] As a result of these discoveries, a modification was made to the procedure which enabled precise control of the temperature and humidity of the inspired air and made it possible to detect patients who normally would have remained undiagnosed.

Although there is agreement that the trigger mechanism for EIB is the abnormal loss of heat and humidity from the airways, some controversy still exists regarding the exact pathogenesis of the process. Basically, there are two theories: The first theory purports that it is primarily the *loss of water* that causes the bronchospasm; it states that the water loss from the bronchial mucosa increases osmolarity in the cells that line the airway which, in turn, release certain mediators that promote bronchoconstriction. This theory contends that although the water loss is the primary trigger, if the air is also cooler (which hastens the drying process) the bronchospasm intensifies.[10,11,12,13] The second theory states that it is primarily the *cooling of the airways* that triggers EIB. The reasoning is that after exercise there is an abnormal, rapid rewarming of the airways; the mechanism of the rapid rewarming has been described as “reactive hyperemia,” i.e., that the abundant, sudden blood flow to the airway vasculature following exercise (in an attempt to normalize the temperature of the airways) causes edema which, in turn, initiates the bronchoconstriction. If the air is also drier, this further contributes to the cooling process (because of the evaporative effect) and intensifies the constriction.[13] Whether a particular theory or

a combination of both explains the process remains unresolved.

In time, other advancements were made in the CACh procedure. It was felt that since the increased ventilation was shown to be the primary trigger of EIB, exercise was no longer required to perform the test.[2,6] As a result, a new methodology, referred to as the Isocapnic Hyperventilation Cold Air Challenge (IHCACh), was developed.[2,6] The test consists of patients hyperventilating dry sub frigid air at a predetermined target ventilation for a fixed period of time, *at rest*. During the procedure, the end-tidal carbon dioxide (Pet_{CO_2}) levels are kept constant by adding carbon dioxide (CO_2) to the breathing mixture, which allows the subject to hyperventilate without becoming dizzy or lightheaded. For the most part, the IHCACh has become the new standard.[14,15,16,17]

Recently, as we shall see, the IHCACh has taken on an even greater importance because of the revised recommendations regarding the definition and diagnosis of asthma.[18] Aside from its ability to detect EIB, a positive CACh, by definition, demonstrates the existence of hyperreactive airways, which is a crucial element in the diagnosis of asthma. (It should be noted that EIB is not a condition considered separate from asthma. Sometimes it is the only symptom that initially indicates the presence of asthma. It has been shown that eventually other symptoms manifest themselves.)[3]

III. Asthma Definition & Diagnosis

A great deal of progress has been made regarding the definition and diagnosis of asthma, making our understanding of the disease far more comprehensive. Traditionally, asthma has been defined and diagnosed primarily by a patient's clinical picture. This placed asthma mostly in the category of being a syndrome (i.e., a collection of a patient's symptoms), rather than a disease, per se. Today, however, there are more specific guidelines.[18] These guidelines state that asthma should be defined as a chronic inflammatory disorder of the airways.[18] The inflammation leads to recurrent episodes of airflow limitation that can reverse either spontaneously or through treatment. This limitation is manifested by symptoms of wheezing, shortness of breath, tightness in the chest and coughing. The symptoms can be caused by a variety of factors which can act either alone or in combination. These include spasm of the smooth muscle, edema, mucus formation, and the more recent discovery of what has been termed *airway remodeling*, i.e., a chronic inflammatory process resulting in irreversible thickening of the airway walls -- a condition which has been associated with the persistence of asthma and may be responsible for those cases where results have been limited, or treatment proven ineffective.[18] Additionally, there is an abnormal increase in the sensitivity to a variety of stimuli which include allergens, environmental irritants, viral infections, cold air and exercise. The heightened sensitivity is referred to as airways *hyperreactivity* or *hyperresponsiveness* and pertains to the bronchoconstriction that occurs under these conditions.[1]

Regarding the diagnosis of asthma, the new standards recommend that the clinician look for three basic components. The first is to make sure that recurrent airflow limitation and its accompanying symptoms are present; the second is to make certain that the symptomatology does not correlate with an alternative diagnosis that could possibly masquerade as asthma (e.g., vocal chord dysfunction, deconditioning, occult cardiac or pulmonary disease, or McArdle's syndrome);[3,19] and finally, it must be demonstrated that the

[1] It should be noted that in most cases airway hyperreactivity is directly correlated with inflammation, but not always. Studies have shown that the relationship can be more complex. [30]

airflow limitation is at least partially reversible or, if not, that hyperreactivity is present.[18] None of these components, however, is individually diagnostic of asthma.

IV. Reversibility / Hyperreactivity

The most common and acceptable method to demonstrate reversible airflow limitation is to measure the response to bronchodilator administration using spirometry. A significant post-dilator response is defined as an improvement of at least a 12% increase in the Forced Expiratory Volume in one second (FEV_1) and a minimum of a 200ml increase in the Forced Vital Capacity (FVC). [20]

In the absence of the ability to demonstrate reversibility, i.e., where patients present with vague or intermittent symptomatology, have normal spirometry, and do not respond to bronchodilators, it is essential to show the presence of airways hyperreactivity to help make a diagnosis.[18] In order to do this, other methods need to be utilized. Rather than using bronchodilators to "open" them up, these patients are *challenged*, or *provoked*, through exposure to agents that induce bronchoconstriction. If the FEV_1 decreases significantly after provocation, the test is considered positive. Normal individuals do not have a significant response to challenge testing.

V. Challenge Methodologies

There are a number of challenge methodologies. These include the CACh, the Methacholine Challenge (MCh), the Histamine Challenge (HCh), and challenge tests performed with other nebulized solutions. The two most popular tests, however, are the CACh and the MCh. The MCh consists of the delivery of aerosolized, increasing concentrations of the drug which are administered to the patient every five minutes (up to a defined maximal dose). The diagnosis of hyperreactivity and its severity is based on the dosage required to decrease the FEV_1 by a defined percentage. The CACh, as we have mentioned before, consists of the inhalation of dry sub frigid air at high minute volumes. If the FEV_1 decreases a specific percentage, the test is considered positive. Although the CACh (which was originally developed to diagnose EIB) and the MCh are both capable of exposing airway hyperreactivity, the underlying mechanisms are not the same because they affect different receptor sites.[21,22,23,24] Therefore, if one method fails to detect airway hyperreactivity, the other should be tried. And even with both, there may still be a gray area of the population that will go undiagnosed. However, if one of the techniques shows airway hyperreactivity, it is not necessary to perform the other. One should also keep in mind that only a CACh can be diagnostic of EIB. A positive MCh, by itself, only proves the existence of airway hyperreactivity and does not confirm a diagnosis of EIB -- this does not mean, however, that someone with a positive MCh cannot have EIB. It simply means they would need to have a CACh, as well.

When done properly, the MCh is a very effective diagnostic tool, but it has some drawbacks. These include the sometimes limited availability of the drug, the uncertain quality of the assays, the fact that it is an unnatural substance, the lack of specificity, the possibility of adverse reactions to the drug (both for the patient and those who administer it) and the need for the drug to be properly mixed and dispensed with precision.[25]

The CACh, when done properly, is also a very effective diagnostic tool and has the benefits of more closely simulating natural conditions and having no side effects.

It should also be mentioned that challenge tests of this type can be used for other things, in addition to the diagnosis of asthma. Among their many uses is the potential to quantify the severity of the hyperreactivity, gauge the effectiveness of various treatment regimens, or monitor the progress of the disease -- in other words, once a positive baseline challenge test has been established, one can do sequential studies and perform trend analysis to evaluate any change in the patient's condition. Other applications include the utility as screening devices for sports activities or occupations where exposure to extreme weather conditions (e.g., cold air), physical stress, or environmental irritants could affect performance.

VI. Sensitivity vs. Specificity

As previously mentioned, the challenge tests should be seen as adjuncts to each other. However, a great deal of debate still exists as to which test is the more discriminating indicator of airway hyperreactivity. The issue relates to the concepts of *sensitivity* and *specificity*, which need to be clearly understood. In the simplest terms, a test's sensitivity is based on its ability to *include* the greatest portion of a population known to have a particular condition, but may inadvertently include some that don't. Conversely, a test's specificity is based on its ability to *exclude* everyone who doesn't have a particular condition, but may include some that do.[15,26] Ideally, one would like a test that is both 100% sensitive and 100% specific, but this is rare because usually the more sensitive one makes a test, the less specific it becomes and vice versa. In the absence of the ideal, the goal is to design criteria that strike the best balance between the two.[2]

With respect to the MCh and the CACh, or any challenge test for that matter, it is crucial to understand that the sensitivity and specificity of these tests are not simply dependent on the substances (e.g., cold air or methacholine) that are utilized, but also on the methodology (e.g., doing the test at rest, or using exercise) that is employed and how it is quantified (spirometry, plethysmography or forced oscillation).[3] Often, these concerns are not taken into account when debating the pros and cons of these tests and can lead to false assumptions regarding their individual merit. Much research needs to be done with respect to these matters. This leads us to the theoretical issues surrounding the CACh.

[2] A good example of this is the administration of bronchodilators to help diagnose asthma. An increase of at least 12% in the FEV_1 and 200ml in the FVC is considered significant.[20] To make the test more sensitive, the criteria may be changed to an increase of 5% in the FEV_1 and 100ml in the FVC. This may very well include some of the population that is normal, making the test less specific. Conversely, the test may be made more specific by changing the criteria to an increase of 20% in the FEV_1 and 300ml in the FVC. A portion of the population that has asthma may be excluded, making the test less sensitive. The American Thoracic Society (ATS) standard is based on extensive testing which includes most subjects who have asthma and excludes most who don't, thereby creating the best balance between *sensitivity* and *specificity*.

VII. Theoretical Considerations

Before we can recommend a specific, generic CACh protocol, we need to consider the following issues:

- Should we use isocapnic hyperventilation or exercise for the CACh?
- What minute ventilation should be used as the hyperventilation target and how long should it be sustained?
- Should we use single or multiple exposure testing?
- After the exposure to cold air is completed, how soon should we begin evaluation testing? How long should the intervals be between successive assessments, and what is the maximum time for a response?
- To maintain a steady Pet_{CO_2}, should we add CO_2 to the inspired air mixture or use a compressed air tank containing 5% CO_2 and air?
- Should spirometry, plethysmography, airway impedance, or some other technique be used as the primary means to quantify a response to the CACh?
- What changes in FEV_1 and sG_{aw} constitute a positive response to Cold Air?

VIII. CACh Methodologies

Basically, there are two CACh methodologies that need to be examined -- the Isocapnic Hyperventilation Cold Air Challenge (IHCACh) and CACh done with exercise. The only other methodology that will be mentioned in this discussion is a technique that employs ambient temperature, dry air isocapnic hyperventilation. Although this technique is not a CACh methodology, it should be examined to help clarify the rationale behind using cold air.

Isocapnic Hyperventilation CACh

In the IHCACh, sub frigid dry air is administered while the patient hyperventilates at approximately 60% of his or her Maximal Voluntary Ventilation (MVV) for a minimum of three minutes, without exercise. To avoid lightheadedness from the hyperventilation (hypocapnia), an isocapnic technique (where the end tidal CO_2 is maintained in the normal range of approximately 40 mmHg) is employed consisting of the patient breathing a mixture of 5% CO_2, balance air.[3] The mixture is virtually dry since the tank is guaranteed to contain only trace amounts of water. In addition, chilling the air to approximately -20ºC causes any remaining water vapor to condense and freeze before reaching the patient. After the cold air exposure, spirometry testing is performed at five-minute intervals over a 20-minute period. If the FEV_1 is significantly reduced, the test is considered positive.

The obvious advantage of the IHCACh is that exercise is not required. That may seem like a contradiction in terms but, as mentioned before, no one has been able to find any biochemical trigger associated with exercise that would account for the bronchoconstriction. The only

[3] Normal Pet_{CO_2} is approximately 5%, indicative of a P_{CO_2} of 40 mmHg.

certain trigger is the increase in ventilation.[3,4,6] Therefore, most people who utilize the IHCACh have made the leap to equate a positive test with EIB.

Ambient Temperature, Dry Air Isocapnic Hyperventilation

The major attraction to using an *ambient temperature*, dry air isocapnic hyperventilation challenge as opposed to the IHCACh is that it eliminates the need to purchase a cold air generator. The reason it is not recommend is because studies demonstrate that there is a **greater response when using cold, dry air as opposed to just dry air at room temperature**.[14,27] It has also been shown that isocapnic hyperventilation using dry air at room temperature elicited only about 2/3 of the bronchoconstriction normally observed when using sub frigid air at similar minute ventilations and that dry air requires a greater minute ventilation to achieve the same degree of obstruction as with cold air.[14] **This shows an increased sensitivity of the CACh at lower levels of ventilation and is particularly important because a significant number of patients with obstructive lung disease cannot increase their ventilation for a sustained period of time.** It would seem, therefore, that the advantages outweigh the need to economize on equipment.

Exercise CACh Testing

The issue of whether exercise should be used in conjunction with CACh testing is a complicated one. As previously discussed, a majority feel that the IHCACh is more than adequate to use in the diagnosis of EIB because an increase in ventilation has been shown to be the primary trigger.[4] However, there are some who feel that unless the CACh is done under exercise conditions, one cannot make a strict diagnosis of EIB. It's hard to argue with the latter, but, based on the evidence, it's easy to agree with the former. Nonetheless, cold air testing with exercise has serious drawbacks.

One of the problems encountered with exercise testing is how to standardize the methodology. Should a treadmill be used, or a bicycle ergometer? The treadmill has the advantage of being more "natural" from the point of view that everyone knows how to walk, but some feel that it produces a less accurate workload than the bicycle. Also, safety factors are another consideration -- if patients have to run in order to achieve their target values, there is always the risk they may slip, or fall, off the treadmill.

Another problem is the issue of what protocol to use. What level of exercise should be achieved and how best do we get there? Some feel that the patients should be exercised to 85% of their predicted heart rates. Others feel that the goal should 80-90% of the patient's maximum workload or V_{O_2} Max (which would require a baseline maximum test), while others feel that the goal should be 60-80% of the patient's MVV for a minimum of three minutes.[3] There is also the question whether a "step" or a "ramp" protocol should be used and in what power increments the workload should increase, or should steady-state testing be employed instead? And how quickly should the patient be brought to their target ventilation, heart rate or workload?

In addition, there are also questions pertaining to the subject's conditioning. If the patient is in poor health, or sedentary, how can they possibly achieve the necessary levels of exercise to trigger a response? Plus, if a patient has a serious, unrelated, or undiagnosed medical problem (e.g. cardiac disease), exercise testing may place them in jeopardy by exacerbating the condition. And even if the patient is basically in good health, or active, there may still be problems in achieving, and *sustaining*, any one of the above-mentioned targets. We know that a maximal exercise test

consists of three phases -- the aerobic, anaerobic and the final phase of metabolic acidosis. Whether the primary goal of the test is to increase the patient's ventilation to 60-80% of their MVV, their heart rate to 85% of predicted or to achieve 80-90% of their maximum workload, these targets are usually only achieved in the metabolic acidosis phase of exercise which, in a normal individual, only lasts a minute or so. Therefore, unless someone is fairly athletic, the ability to sustain a period metabolic acidosis under exercise conditions for a specified period of time would be difficult, at best.

In conclusion, it appears that the problems in standardizing an exercise protocol that would satisfy everyone are insurmountable. Therefore, it is recommended that CACh testing be done using the IHCACh technique. There are three main reasons for this: 1) increased ventilation appears to be the primary trigger of EIB; 2) the test is standardized for everyone and is highly quantifiable; and 3) it is safer than exercise.[4] Nonetheless, in those instances where it has been decided that exercise is desired, for whatever reason, it can be handled in one of two ways: each laboratory can establish its own standard; or, exercise CACh testing can be customized for the individual patient to more closely approximate their type and level of physical activity.

IX. Target Ventilation: What Rate & Duration?

A review of the literature demonstrates that known asthmatics need to sustain a minimum of 60% of their MVV for no less than three minutes in order to achieve the maximum response to cold air.[14] It has been shown that there is little gained by increasing the target ventilation above 60% of the MVV or sustaining the ventilation beyond three minutes.[14]

The target ventilation can be obtained in one of two ways. It can be calculated directly by making an actual measurement of the patient's MVV and multiplying it by 60%, or one can measure the patient's FEV_1 and multiply it by 24, which also results in 60% of the MVV. Ideally, one should measure the MVV directly, but there may be instances where some patients are so hyperreactive that the mere act of performing an MVV maneuver can trigger significant bronchospasm. In those instances, if it is possible to document the degree of the bronchospasm without endangering the patient, and if it can be shown to be equal to that of a positive test, the data should be recorded and the procedure considered diagnostic of hyperreactive airways disease.

X. Single vs. Multiple Exposure

Should one use a single exposure (SE) or multiple exposure (ME) protocol for the CACh procedure? Each has advantages and disadvantages. The SE protocol consists of administering only one, three-minute dose of cold air at the patient's target minute volume, while the ME protocol consists of exposing the patient to multiple three-minute doses of cold air at graduated ventilatory increments, typically 10, 20, 40 and 80 percent of the patient's MVV.[15]

The obvious advantages of the SE protocol are that it takes considerably less time and consumes much less gas than the ME protocol. After the exposure to cold air, spirometry is performed at five-minute intervals until a significant response is measured, over a maximum of twenty minutes. If we include cold air acclimatization, cold air exposure and the evaluation period, the test should take no longer than thirty minutes. Using a single exposure of cold air and an "H" cylinder of gas, one could conceivably perform three to four tests from one tank of gas.

Seemingly, the only disadvantage of the SE protocol is that it cannot accommodate those patients who are incapable of maintaining their target ventilation (due to suspected bronchospasm, rather than discomfort or lack of coordination). This can be dealt with in one of two ways. The first is to stop the test and proceed to the ME protocol once the patient has recovered, as demonstrated by a return to the baseline FEV_1 value. The second is to simply allow the patient to hyperventilate at the lower level of ventilation for the remaining time, document the level at which it occurred and continue with the rest of the procedure (although a negative test should not be accepted as definitive). [The only time this is impractical is if patients exhibit such distress that they are unable to complete the cold air exposure. In such a case, the cold air exposure should be terminated immediately and, if possible, spirometry should be performed to document the response. This situation is extremely rare, however, because it has been shown that the bronchospasm normally occurs post-challenge, during the rewarming period (usually five to fifteen minutes after exposure), not during the exposure to cold air.][13,3]

The primary advantage of the ME protocol seems to be that one can identify the exact level of ventilation that provokes bronchospasm. This may help the clinician advise patients as to the degree of activity that they may be able to perform before they run into difficulty. Another advantage is that patients who are unable to comply with the relatively high ventilatory requirements of the SE protocol may be evaluated at lower minute volumes due to the graduated ventilatory increments of the ME protocol.

The primary disadvantages of the ME protocol are that it consumes a substantial amount of gas and takes much longer to complete. If one has to administer four doses of cold air, it is possible that one tank of air may not be enough and the total test time could take hours. Even if a positive response is obtained in as little as two doses, the gas and time requirements are twice that of the SE protocol.

In addition, the total test time depends on the interval between doses, which is at least 5 minutes. It has been shown that the time to peak response to cold air exposure may be as long as fifteen minutes.[3,13] Therefore, if the interval is as short as five minutes, the ventilatory level that produced the bronchospasm may be incorrectly identified due to the overlap of the earlier response. Lengthening the interval to avoid this problem may lead to much longer test times. Furthermore, in order to separate the influence of individual doses, the patient must recover completely from the effect of the previous dose as demonstrated by a return to the baseline FEV_1 value. This process may take from 30 to 60 minutes.[3,4,13] If the precise level of ventilation that produced the bronchospasm must be identified, one must wait for the patient to recover between doses, possibly extending the ME protocol test time to several hours, which is highly impractical.

Another complication with the ME technique is that it may trigger a “refractory period”. In essence, a refractory period is the time during which the bronchospastic response is lessened by exposure, or repeated exposure, to a particular stimulus. There is strong evidence that repeated hyperventilation maneuvers with exercise, cold air, dry air and, in some cases, even ambient air can create a refractory period.[26,41,42,28] Whether this is due to the release of catecholamines or the depletion of certain mediators is not completely understood. In any case, regardless of the fact that the ME technique is begun at low levels of ventilation, it is possible that repeated maneuvers may attenuate the degree of bronchospasm and underestimate the true level at which it occurs. Therefore, in an attempt to precisely determine

the level of ventilation at which significant bronchospasm is triggered, the effect of the refractory period may actually cause the incorrect identification of too high a level.

A final issue concerns whether one technique is more precise than the other. Studies have shown that both are equally diagnostic, i.e., quantifying the decrease in FEV_1 at a specific time period does not appear to be any less exact than quantifying the decrease in FEV_1 at a specific level of ventilation. [22]

All things considered, the SE protocol is recommended because of its economy of time and gas consumption as well as the fact that it rules out the possibility of a refractory period.

XI. Titration of CO_2 vs. 5% CO_2 & Air Mixture

Isocapnic hyperventilation refers to the concept of maintaining a patient's baseline Pet_{CO_2} as ventilation is increased. The main purpose of this technique is to keep patients from becoming dizzy while trying to reach their target ventilation and to prevent the bronchoconstriction that may be caused by the effect of severe hypocapnia. This can be done in one of two ways: 1) monitor the patient's Pet_{CO_2} with a capnograph or exercise testing system and add the necessary amount of CO_2 to maintain the baseline Pet_{CO_2} or 2) have the patient breathe from a tank that contains a mixture of 5% CO_2, balance compressed air (5% CO_2, 21% O_2, 74% N_2).

In the first instance, the difficulty with trying to maintain a patient's Pet_{CO_2} while adding CO_2 is the same problem that is incurred when trying to maintain a constant F_{IO_2} for a patient with a nasal cannula -- the ever-changing tidal volume dilutes the gas unevenly and doesn't allow for a uniform concentration. When trying to maintain a consistent Pet_{CO_2}, the problem is even further complicated by the fact that the patient has to breathe faster in order to maintain a high minute volume. Trying to keep pace with this is difficult and distracts the operator from coaching the patient to the best of his or her ability.

When using the 5% CO_2, balance air mixture, the studies have shown that people who require target minute ventilations in the range of 40-105 lpm (which includes the majority of the test population) will not significantly veer from their baseline Pet_{CO_2} (35-45 mmHg).[17] Even at the unusually high target ventilation of 150 lpm (which would be required by patients with an FEV_1 of close to 6.00L) the Pet_{CO_2} will not go below 30 mmHg. Conversely, at target ventilations of only 25 lpm (which would be required by patients with an FEV_1 of 1.00L) the Pet_{CO_2} will not exceed 50 mmHg. **These parameters are well within a range that would not cause an abnormal reaction and, therefore, it is highly recommended that the 5% CO_2, balance air mixture be the method of choice**.[6]

XII. Time Intervals

When using the SE protocol, a minimum of a three-minute exposure to cold air is required.[14] However, there are other issues of time to be considered: 1) how soon after the patient is exposed to cold air should the spirometry testing begin, 2) how long should the time intervals between successive assessments be and 3) what should the maximum time be to test for a response to cold air?

Regarding the first issue, there are basically two choices: to test immediately after the exposure to cold air or to wait for a specified time period.

Although there is nothing wrong with testing immediately after the CACh, it's been shown that if significant bronchospasm occurs, it usually reaches its peak five to fifteen minutes after exposure, during the rewarming period, i.e., that it is not a transient phenomenon.[14,28,4] Based on this information, it is recommended that testing should begin five minutes after exposure.

Concerning the time intervals between successive spirometry evaluations, studies show that 5-minute intervals are optimum. It was demonstrated that there is no difference in the maximum decrease in FEV_1 if the testing intervals were less than five minutes or if continuous assessment were performed.[14,9]

Finally, with regard to the length of time the patient should be tested, since the maximum response usually occurs from five to fifteen minutes after exposure, it is recommended that the post-challenge testing run no longer than twenty minutes. The post-challenge testing period is terminated once the designated patient response to cold air is observed. Continuing the testing might endanger the health of the patient resulting in such severe bronchospasm that reversal might be difficult and could lead to an emergency situation.

XIII. Spirometry, Plethysmography or FOT

As previously mentioned, in order to properly evaluate the sensitivity and specificity of a challenge test, one must consider how accurately it is measured. If the quantification process is inaccurate, or incomplete, the legitimacy of the test comes into question. Basically, in an effort to gauge the response to CACh's, parameters are sought that will quantify changes in airflow reduction resulting from increased airway resistance. Simply put, airway resistance is a measure of the caliber (or width) of the airways; the caliber of the airways can be effected by occlusion (e.g., mucous formation), edema, bronchoconstriction caused by contraction of the smooth muscle around the airway, airway collapse caused by changes in lung compliance (as in COPD), or any combination of the above. [30,31]

The parameters to be considered for the measurement of airway caliber are the FEV_1, airway resistance (R_{aw}), specific resistance (sR_{aw}) and specific conductance (sG_{aw}), for which the clinician may utilize methods such as spirometry, plethysmography or forced oscillation.

FEV_1

The FEV_1 has long been the standard parameter for gauging airway obstruction and the response to the administration of bronchodilators and bronchoconstrictive agents. The parameter is taken from the Forced Vital Capacity maneuver and, if there is no problem with patient coordination, it is highly reproducible. There is a general misconception, however, that the FEV_1 is a direct reflection of the measurement of R_{aw}. Although the parameters are similar, there is a significant difference.[30]

The primary difference is the fact that the FEV_1 (which is a measure of flow) is not a pure measurement of R_{aw} because it is a forced maneuver, i.e., the amount of air one exhales in one second is not only dependent on the amount of resistance in the airway, it is also dependent on patient effort and driving pressure. This relationship is stated by Ohm's Law: Resistance = Driving Pressure / Flow. [30] Therefore, changes in airway resistance are not necessarily manifested as equivalent changes in flow, i.e., the FEV_1 measurement, by itself, cannot discriminate between the effect of airway resistance and driving pressure. As a result, false conclusions

may be drawn about the presence, or degree, of airway obstruction.[30,31]

With regards to the above, it is possible that one might see little or no change in the FEV_1 until bronchoconstriction becomes significant, while changes in airway resistance are apparent. The reason is that unless someone is in the endstage of pulmonary disease, the amount of driving pressure available to them normally exceeds the requirements to reach maximum flow.[31] As a result, an increase in the driving pressure may overcome a larger resistance while the flow remains unchanged. Therefore, a narrowing of the airways may not interfere with the amount of air that can be exhaled in one second, but it may very well increase, geometrically, the resistance of the airway. This is often manifested clinically where patients claim to feel better after bronchodilator administration and no changes are seen in the FEV_1, yet changes are seen in other indicators which are direct measurements of airway resistance (e.g. R_{aw}).[32] We can see, therefore, that although the FEV_1 is a highly reproducible test, it lacks a certain degree of sensitivity as an indicator of pure airway resistance due to its dependency on patient effort and driving pressure.[31]

Another complication with the FEV_1 is the fact that the deep breath taken before the forced exhalation may effect bronchomotor tone. This may cause bronchodilation or, in rarer cases, bronchoconstriction.[29]

Nonetheless, on the positive side, the FEV_1 still captures a large majority of obstructive airway problems. In addition, it is easy to perform for most people, it is highly reproducible, and the spirometers used to measure it are standard equipment in all laboratories.

R_{aw}, sR_{aw} and sG_{aw}

An alternative, or adjunct, to the FEV_1 is a parameter called airways resistance (R_{aw}), which is measured in a body plethysmograph. This measurement differs from the FEV_1 in that it is not forced and is measured while the patient performs a gentle panting maneuver close to his or her Functional Residual Capacity (FRC). The maneuver can also be done using resting breathing. The panting maneuver keeps the glottis and vocal cords open and helps to minimize the effect of oropharyngeal resistance. The resting maneuver (usually done if there is a problem with panting) results in somewhat higher resistance measurements because of the oropharyngeal component. Even though the resting maneuver is more "natural", it is not recommended because the large oropharyngeal component compromises the sensitivity to acute changes in the intrathoracic airways.

During the R_{aw} maneuver, pressure measurements must be made in order to calculate resistance. This is accomplished by a patient panting gently against a closed shutter. However, because these techniques are not forced maneuvers, and because they are made close to FRC, patient effort and the influence of elastic recoil on airflow is greatly minimized, leaving us with a purer measurement of airway resistance.[30,31]

The measurement and interpretation of R_{aw}, on the other hand, are not as easy as they may appear. The reason for this is that R_{aw} also has a relationship to lung volume. Normally, as we breathe deeper and increase our lung volume, our airways distend and resistance decreases (Figure 1). Therefore, patients who develop chronic increases in resistance due to airway obstruction will have a tendency to increase their FRC and breathe higher up in their lung volumes in an effort to maintain normal resistance. In those instances, if a single, absolute measurement of R_{aw} is made without accounting for the compensation

in lung volume, it is possible that the value for R_{aw} may be normal despite the fact that the patient is significantly obstructed. There is a solution to this problem, however, and it lies in the measurements of specific conductance (sG_{aw}) and specific resistance (sR_{aw}). These parameters account for the resistance at specific lung volumes and help to expose abnormalities that are sometimes masked by normal measurements of R_{aw}. [32]

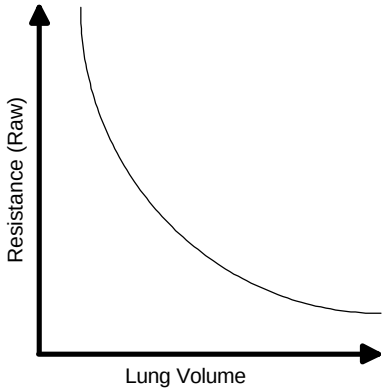

Figure 1

Briefly, if we look at R_{aw} (Figure 1), it is hyperbolically related to lung volume and *decreases* as volume increases. Conductance (G_{aw}), which is the mathematical reciprocal of R_{aw} ($1/R_{aw}$), is linearly related to lung volume (Figure 2) and is easier to conceptualize and quantify than the R_{aw} measurement. G_{aw}, in opposition to R_{aw}, *increases* as lung volume increases. Specific resistance (sR_{aw}) and specific conductance (sG_{aw}) simply account for the lung volume at which these measurements are made. Therefore, in the above instance where it was seen that chronic obstruction could manifest as a normal R_{aw} measurement, the condition would be exposed if sR_{aw} and sG_{aw} were also used, i.e., sR_{aw} would go up and sG_{aw} would go down, revealing that at the higher lung volumes the resistance is abnormal.[32] Another advantage of these measurements is that, unlike the FEV_1 (where different predicteds are required for age, height and sex), a single reference value can be used for everyone.

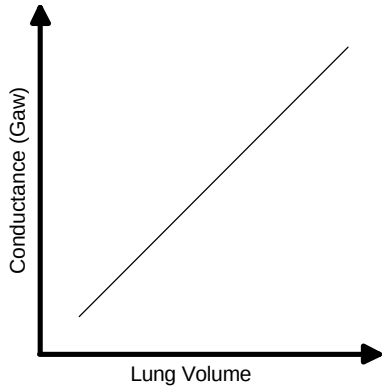

Figure 2

The critics of R_{aw}, sR_{aw} and sG_{aw} have some valid points, however, most of which are based on the technical and procedural aspects of the maneuvers, not necessarily the theoretical basis of the measurements. One criticism is the lack of plethysymographs as standard equipment in most laboratories. Another is the need to calibrate multiple transducers and check for frequency response, thermal stability and leaks. In addition, improper panting techniques, excessive pressure fluctuations and signal drift can lead to erroneous measurements. Other criticisms include the choice and application of various reference values (which affect interpretation), the sometimes prohibitive cost of plethysmographs, and the fact that some feel the maneuvers are not as reproducible as the Forced Vital Capacity (FVC), which yields the measurement of FEV_1. [33]

Nevertheless, from a theoretical point of view, it appears that R_{aw} and its related measurements (when done properly) are more sensitive indicators of airway obstruction than the FEV_1. However, as discussed, the FEV_1 is the easier of the two to perform, is the standard, is highly reproducible, captures a large majority of obstructive airway cases and requires less complicated equipment. Those who are skilled operators of the plethysmograph may argue the "ease of performance" issue but, practically speaking,

there seem to be less skilled operators of the plethysmograph than there are of spirometers. A combination of both FEV_1 and R_{aw} measurements would seem ideal.

Forced Oscillation Technique (FOT)

The Forced Oscillation Technique (FOT), an alternate means of measuring airway resistance (impedance), although a recent innovation, is a technique that was originally developed by Dubois et al in 1956.[34] The primary reason for its lack of use until this time has been the fact that although the equipment setup and procedure are relatively easy, the mathematical calculations are very complex. With the advent of computers, however, that is no longer an issue.

Of all the techniques we have discussed so far, the FOT is, by far, the easiest. The patient breathes normally on a mouthpiece for approximately fifteen to twenty seconds. There are no deep inspirations or forced exhalations and, therefore, there are no effects on bronchomotor tone, nor are patient effort and driving pressure, and their influence on R_{aw}, an issue.

Rather than the patient performing a forced expiratory maneuver and providing the power, the FOT device, so to speak, provides the driving force. The machinery consists of a loudspeaker with pressure and flow transducers. The loudspeaker (power source) emits a signal into the lungs. These waves are generated over a range of frequencies, typically ranging from 5Hz to 35Hz. Based on certain models of the lung (Mead's equation of motion of the lung), the "echoes" of the pressure and flow measurements that are reflected back provide a measurement of *Total Respiratory Resistance*. Total Respiratory Resistance includes airway resistance, chest-wall resistance and tissue resistance. It is believed that through careful analysis it is possible to separate the various resistances (the central airways from the peripheral airways) based on the response to the excitation at various frequencies.

The drawbacks associated with the FOT are few, but they are significant. The biggest problems have to do with the terminology and interpretation of the data. The concept is described using engineering terminology such as Impedance, Resistance, Reactance, Inertance and Capacitance. Without a background in the physical sciences, trying to conceptualize how these terms relate to the lung is often confusing Furthermore, the parameters are interrelated and dependent on frequency. Occasionally, this may cause difficulty in the analysis of the results. In addition, because the technique is based on a specific lung model, if that model does not apply to the physiology (e.g., glottic or pathologic changes), the results may be profoundly distorted.

In many studies it has been shown that the FOT is as sensitive as the plethysmograph method for detecting airway resistance. Other studies that have compared the FOT, spirometric and plethysmographic methods have revealed the FOT to be the most sensitive indicator of R_{aw}, with sG_{aw} and FEV_1 being second and third, in that order.[35,36,37,38,40]

The FOT appears to have great promise because of its ability to test patients while requiring little cooperation. However, it is very new, and before it becomes a standard a great deal will have to be learned about its advantages and limitations.

What Change Constitutes a Positive Reponse?

Although there are still no American Thoracic Society (ATS) standards for CACh's, the majority of the literature supports the criterion of a 10% to 15% decrease in FEV_1 as the cutoff point for a positive CACh.[28,3,4] The consensus is that

anything less than 10% would make the CACh too sensitive and greater than 15% would make it too specific. For sG_{aw}, the equivalent would be a 35-40% decrease.[39]

What is the Best Indicator of a Positive Response?

In an effort to find the best indicator to evaluate the response to the CACh testing, the conclusion is that no one parameter is best. It would seem that using a combination of FEV_1, sG_{aw} and sR_{aw} would be ideal (the FOT still being too new to be considered a standard). In fact, there is evidence to show that the FEV_1 in combination with sG_{aw} provides the optimal means of assessment.[29] However, we also need to be practical. Most laboratories are not equipped with body plethysmographs -- and even though a combination of parameters seems preferable, if only one parameter had to be selected, the choice would be the FEV_1. The reasons are obvious. It is the standard, it is highly reproducible, and all laboratories have some type of spirometer capable of making the measurement. However, in those instances where the testing laboratory has access to both pieces of equipment, it is highly recommended that both spirometry and plethysmography be utilized.

XIV. Summary

Issue #1: Should isocapnic hyperventilation or exercise be used ?

Answer: Isocapnic Hyperventilation

Reasons:
- Increased ventilation is the primary trigger of bronchospasm.
- The IHCACh is easily quantifiable.
- There is no known biochemical trigger attributed to exercise.
- Exercise protocols are too numerous and varied for the majority to come to a consensus.

Issue #2: What percentage of the MVV should be used as the target ventilation and how long should the target ventilation be sustained?

Answer: Sixty percent of the MVV for a period of three minutes

Reasons:
- Less than 60% of MVV is not enough, and more than 60% does not add substantially.
- Most people can sustain 60% of their MVV; 80% would be difficult for many.
- Less than three minutes at the target ventilation is not enough; more makes little difference and may be difficult to sustain.

Issue #3: Should a single exposure (SE) or Multiple Exposure (ME) protocol be employed?

Answer: **Single Exposure**

Reasons:
- It takes less time to perform.
- It consumes much less gas.
- No risk of "blunting" of the response due to repeated exposures to cold air.
- A strict ME protocol needs to allow a 30-60 minute recovery period between trials.
- Quantifying the response by measuring the drop in FEV_1 at a specific time period is just as accurate as measuring the drop in FEV_1 at a specific level of ventilation.

Issue #4: How soon after cold air exposure should we begin to test for a response?

Answer: **Five minutes**

Reasons:
- The response to cold air exposure is not a transient phenomenon.
- Bronchospasm occurs during the rewarming period, which peaks at 5-15 minutes post-challenge.
- No difference was shown in % maximum decrease in FEV_1 in intervals less than five minutes.

Issue #5: How long should we wait between successive evaluations?

Answer: **Five minutes**

Reasons:
- There is no difference in % maximum decrease in FEV_1 in intervals less than five minutes, or no intervals at all.
- It allows for a small rest period between flow-volume loop trials.

Issue #6: When is the post-challenge evaluation complete? Over what period of time should we test?

Answer: **Twenty minutes or until the designated response is reached.**

Reasons:
- The peak reaction occurs between 5 and 15 minutes post-exposure. The test should run twenty minutes to be certain that the enough rewarming time is provided for a response.
- No longer than is required to elicit the designated response to minimize patient risk due to severe bronchospasm.

Issue #7: What parameter should we use to quantify the response?

Answer: FEV_1, if only one parameter is possible, but also recommend sG_{aw}.

Reasons:
- It's the standard.
- It's highly reproducible.
- Many labs don't have body plethysmographs and cannnot measure sG_{aw}.
- The FOT is promising, but more testing is needed before it becomes a standard.

Issue #8: What percent decrease in FEV_1 is significant?

Answer: 10-15%

Reasons:
- Less than 10% makes the test too sensitive.
- More than 15% makes the test too specific.

Issue #9: Should we titrate CO_2 into the inspired air or use a mixture of 5% CO_2, balance air?

Answer: 5% CO_2, balance air

Reasons:
- It maintains the Pet_{CO_2} better.
- It frees up the technologist to coach patient.
- There is a safety factor. Improper monitoring while adding CO_2, especially with children, may lead to serious (and sometimes permanent) injury.

XV. Single Exposure Protocol

Section I: When not to perform the test

- If the patient is suffering from a cold, or any type of respiratory infection, the test should be postponed until the patient is symptom free.
- If the patient is unable to coordinate the test or perform reproducible flow-volume loops, the test should not be performed.
- If the patient's baseline FEV_1 is 65% of predicted or below or the FEV_1/FVC ratio is below 70%, the test should not be done. (Patients with advanced obstructive pulmonary disease, for safety reasons, are not good candidates for bronchial challenge test procedures and are excluded due to the substantial reduction in their baseline spirometry measurements. Provoking these patients may lead to acute, severe bronchospasm that may require emergency intervention.)
- If a physician order or prescription, which is required for the test, is not available.

Section II: Equipment and supplies

Required:

- Spirometer to measure flow-volume loops
- Device to measure minute ventilation (V_E) (Target meter or exercise system)

Recommended:

- Pulse Oximeter
- Crash Cart
- Means of delivering a bronchodilator, when necessary
- Device to monitor end-tidal CO_2 (Pet_{CO_2})

Section III: Pretest preparations

- A consent form detailing the test and the possible complications should be signed by the patient or guardian.
- The patient should refrain from smoking at least 2 hours prior to the test.
- The patient should refrain from consuming any food or drink that contains a significant amount of caffeine, e.g., coffee, cola or chocolate for at least 2 hours prior to the test. (Caffeine can have a bronchodilatory effect).
- The patient should refrain from strenuous exercise on the day of the test.
- Antigen and occupational exposures should be avoided for at least 24 hours.
- Based on the 1992 AARC Clinical Practice Guidelines for Bronchial Provocation, the following medications should be withheld before testing**: ***
  - Beta 2 - adrenergic aerosols -- 12 hours.
  - Anticholinergic aerosols -- 12 hours
  - Disodium cromoglycate -- 8 hours
  - Oral beta 2- adrenergic agonists -- 12 hours
  - Theophyllines -- 48 hours
  - H1- receptor antagonists -- 48 hours
  - Antihistamines -- 72-96 hours
- If the patient is on a beta blocker, the physician should be consulted to see if the beta blocker can be discontinued and, if so, for how long before the test. ***** (Beta blockers may induce bronchospasm, especially in asthmatics.)
- If the patient is on corticosteroids, inhaled or oral, when possible, the drug should be discontinued early enough that the effect at the time of the test is minimal. The length of time should be determined by the physician. *****

*** All changes in medication dosage, <u>including withholding</u>, should be reviewed with the prescribing physician.**

Section IV: Pretest instructions

Before the testing begins, patients should be shown:

- How to perform an acceptable flow-volume loop.
- How to perform a Maximum Voluntary Ventilation (MVV) maneuver.
- How to follow the target ventilation device.

They should be told that:

- They will be required to do three acceptable, repeatable baseline flow-volume loops.
- After the baseline loops, they will be breathing dry frigid air for approximately 5 minutes, during which time they will need to increase their ventilation significantly. Reassure the patient they will not experience any lightheadedness during the isocapnic hyperventilation phase because their CO_2 is replenished by the inspired 5% CO_2 mixture.
- During the test, their oxygen levels will be monitored with a pulse oximeter, as a precaution.
- Following cold air breathing they will be required to perform acceptable loops every five minutes for up to 20 minutes.
- You are prepared to administer a bronchodilator during, or after, the test in the event they go into bronchospasm.
- Emergency equipment is close at hand.

Section V: How to perform the test

- ✓ If at any time during the procedure, a patient becomes so symptomatic that he or she feels that continuing is impossible, terminate the test and administer a bronchodilator immediately.

- ✓ If a pulse oximeter is available, use it throughout the test to monitor the patient. If the Sp_{O_2} falls below 85%, discontinue the procedure.

1. Explain the requirements of the test to the patient.

2. Have the patient perform several spirometry maneuvers. A minimum of two reproducible baseline flow-volume loops is required. If the patient is incapable of this due a lack of coordination or effort, there is no point to continue the test, because there is no valid means of comparing the pre- and post-challenge performance. If the loops are acceptable but appear smaller with each successive effort, resulting in a drop in the FEV_1 of 10% or more, this usually is indicative of highly sensitive airways and should be considered the equivalent of a positive test. If this occurs, document the results and terminate the test.

3. Calculate 60% of the patient's MVV to be used as the target during hyperventilation. In some instances the patient may not be able to perform the actual MVV maneuver because the effort itself may induce bronchospasm. In this case, multiply the patient's FEV_1 by 24 to be used as the target value.

4. Seat the patient and adjust the Turboaire Challenger (TAC) so that the patient is comfortable while breathing through the mouthpiece.

5. Explain to the patient that the metal portions of the TAC generate extremes of temperature during the test. Instruct the patient not to touch either end.

6. Turn on the 5% CO_2, balance air supply tank and adjust to it 100 psi. Operation at a higher pressure does not provide an appreciably lower temperature.

7. Place the patient on the TAC, block their nose and allow them to breath normally for approximately 1 minute. This gives the TAC time to cool down to operating temperature (approximately -20º C) and allows the patient to acclimate to breathing the cold air.

8. At the conclusion of this initial period, have the patient begin to hyperventilate at the target ventilation. Remind the patient to look at the target ventilation meter. Let them know that they need to maintain the target value until instructed otherwise. They should also be

informed that the target ventilation can be achieved by breathing faster, or deeper, or a combination of both. This is reassuring to the patient because some people are not capable of breathing deep *and* fast - they can only do one or the other.

9. If a patient exceeds the target ventilation, it may enhance the effect of the cold air challenge (CACh) response, however, there is a risk that the increased work of breathing may fatigue the patient and result in a premature termination of the test. Therefore, it is recommended to adhere to the target value.

10. It may take some time before the patient achieves his or her target minute ventilation. Be patient. There is a learning curve until the patient becomes comfortable with the best way to maintain the elevated ventilation. Once they're able to sustain it, that's the point at which the clock should begin, i.e., from that point on, the patient should maintain that level of ventilation for a minimum of 3 minutes.

11. After completing the required time at the target ventilation, remove the patient from the TAC. Make sure you have tissues at hand because, in most cases, the patient will have an abundance of saliva. Before you remove the patient from the mouthpiece, place the tissues just below their mouth. Also, have some extra tissues handy to absorb the saliva that may drip from the mouthpiece once the patient comes off the TAC. (Note: Remember to turn off the TAC supply tank.)

12. At this time, you should mark the start of a 5 minute waiting period until the first series of post-challenge flow-volume loops. It is not necessary to perform flow-volume loops immediately after the test is done. It has been shown that the bronchospasm associated with the CACh is not a transient phenomenon. However, if one insists on doing flow-volume loops immediately after the test, no harm will be done. Normally, the only occasion where one would consider performing a flow-volume loop immediately after a CACh is when a patient "tightens up" during the test and the procedure is terminated prematurely. This usually means that the patient is extremely sensitive to cold air. In such a case, it should be documented immediately by performing a flow-volume loop, unless the patient is in such distress that they need to be attended to. **Patient safety always comes first.**

13. Repeat flow-volume loops every 5 minutes until the change that your lab considers significant (usually a 10-15% drop in FEV_1) is reached, up to a 20 minute limit. If that value is attained within the twenty minute post-exposure period, normally, the test is considered complete. However, some laboratories prefer to document the maximum drop in FEV_1 before they conclude the test. From a scientific point of view this may be understandable, but one could be putting the patient in jeopardy. It's possible that the patient could go into such severe bronchospasm, that reversal might be difficult and could lead to an emergency situation.

XVI. References

1. Deal EC, McFadden ER, et al. Hypernea and heat flux: initial reaction sequence in exercise-induced asthma. *J Appl Physiol* 46:476-483, 1979.
2. McFadden ER. Exercise induced asthma. *Amer J Med* 68:471-472, 1980.
3. McFadden ER Jr. Exercise-induced airway obstruction. *Clin Chest Med* 16(4):671-682, 1995.
4. McFadden ER JR, Gilbert IA. Exercised-induced asthma. *N Engl J Med* 330: 1362-7,1994.
5. Chan-Yeung MMW, Vyas MN, Grzybowski S. Exercised-induced asthma. *Amer Rev Repir Dis* 104:915, 1971.
6. McFadden ER, Ingram RH. Exercise-induced asthma. Observations in the initiating stimulus. *N Engl J Med* 301:763,1979.
7. Deal EC Jr, McFadden ER Jr, Ingram RH Jr, Jaeger JJ. Esophageal temperature during exercise in asthmatic and non-asthmatic subjects. *J Appl Physiol* 46:484, 1979.
8. Strauss RH, McFadden ER, Ingram RH, Jaeger JJ. Enhancement of exercised induced asthma by cold air. *N Engl J Med* 297:743-747, 1977.
9. Strauss RH, McFadden ER, Ingram RH, Deal EC, Jaeger JJ. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. *J Clin Invest* 61:433-440, 1978.
10. Anderson SD, Smith CM. Osmotic challenges in the assessment of bronchial hyperresponsiveness. *Am Rev Respir Dis* 143(3 Pt 2) :S43-46,1991.
11. Anderson SD, Daviskas E. The airway microvasculature and exercise induced asthma. *Thorax* Sep; 47(9):748-752,1992.
12. Anderson SD. Issues in exercise-induced asthma. *J Allergy Clin Immunol* Dec; 76(6): 763-772,1985.
13. Tan RA, Spector SL. Exercise-induced asthma. *Sports Med* Jan: 25(1):1-6,1998.
14. Assoufi BK, Dally MB, Newman-Taylor AJ, Denison DM. Cold air test: a simplified standard method for airway reactivity. *Bull Eur Physiopathol Res* 22:349-357, 1986.
15. Eliasson, AH, Phillips YY, Rajagopal KR. Sensitivity and Specificity of Bronchial Provocation Testing: An Evaluation of Four Techniques in Exercise-Induced Bronchospasm. *Chest* 102:347-355,1992.
16. O'Byrne PM, Ramsdale EH, Hargreave FE. Isocapnic Hyperventilation for Measuring Hyperresponsiveness in Asthma and in Chronic Obstuctive Pulmonary Disease. *Am Rev Respir Dis* 143:1444-1445, 1991.
17. Phillips Y, Jaeger J, Laube B, Rosenthal R. Eucapnic Voluntary Hyperventilation of Compressed Gas Mixture. *Am Rev Respir Dis* 131: 31-35.1985.
18. National Asthma Education and Prevention Program. *Expert panel Report 2: Guidelines for the Diagnosis and Management of Asthma.* National Institutes of Health pub no. 97-4051. Bethesda, MD,1997.
19. McFadden ER Jr, Zawadski DK. Vocal Cord dysfunction masquerading as exercise-induced asthma. A physiologic cause for "choking" during athletic activitites. *Amer J Respir Crit Care Med* 153(3): 942-947, 1996.
20. AARC Clinical Practice Guideline. Spirometry Update. *Respir Care* 41(7):629-636,1996.
21. Filuk RB, Serrette C, Anthonisen NR. Comparison of responses to methacholine and cold air in patients suspected of having asthma. *Chest* 95(5): 948-952, 1989.
22. Modl M, Eber E, Steinbrugger B, Weinhandl E, Zach MS. Comparing methods for assessing bronchial responsiveness in children: single step cold air challenge, multiple step cold air challenge, and histamine provocation. *Eur Respir J* 8(10): 1742-1747, 1995.
23. Roach JM, Hurwitz KM, Argyros GJ, Eliasson AH, Phillips YY. Eucapnic voluntary hyperventilation as a bronchoprovocation technique. Comparison with methacholine inhalation in asthmatics. *Chest* 105(3) 667-672, 1994.
24. Steinbrugger B, Eber E, Modl M, Weinhandl E, Zach MS. A comparison of a single- step cold-dry air challenge and a routine histamine provocation for the assessment of bronchial responsiveness in children and adolescents. *Chest* 108(3): 741-745, 1995.

25. Wanger J. Pulmonary Function Testing - A Practical Approach. *Williams and Wilkins* - Second Edition, Copyright 1996.

26. Scharf SM, Heimer D, Walters M. Bronchial Challenge with Room Temperature Isocapnic Hyperventilation: A Comparison with Histamine Challenge. *Chest* 88:586-593, 1985.

27. Deal EC, McFadden ER, et al. Role of respiratory heat exchange in production of exercise-induced asthma. *J Appl Physiol* 46:467-475, 1979.

28. Blackie SP, Hilliam C, Village R, Pare PD. The time course of bronchoconstriction in asthmatics during and after hyperventilation. *Am Rev Respir Dis* 142(5): 1133-1136, 1990.

29. Wanger J. Bronchial Provocation Testing: Relationship to Airway Mechanics. *Amer Assoc for Resp Care: Diagnostics Bulletin* No.2, Summer 1996.

30. Snow MG. Measurement of Airway Resistance. *Jour for Resp Care Prac* Oct/Nov 1993.

31. Snow MG. Use of Plethysmographic Measurements of Resistance and Conductance to Assess Reversible Airways Obstruction. *Amer Assoc for Resp Care: Diagnostics Bulletin* No.2 , Summer 1996.

32. Snow M. Pulmonary Function Interpretation Strategies. *AARC Diagnostics Bulletion* Issue NO.2, 1998.

33. AARC Clinical Practice Guidelines-Body Plethysmography. *Resp Care*. 39(12)1184-1190, 1994.

34. Dubois AB, Brody AW, Lewis DH,etal. Oscillation mechanics of lungs and chest in man. *J Appl Physiol*. 8:587-594, 1956.

35. Ducharme F, Davis GM. Respiratory resistance in the emergency department. *Chest* 113: 1566-1572, 1998.

36. Gimeno F, Postma DS, Van Altena R. Comparison of three normal breathing techniques to assess reversibility of arway obstruction. *Ann Allergy* 69(6): 513-517, 1992.

37. Schmekel B, Smith HJ. The diagnostic capacity of forced oscillation and forced expiration techniques in identifying asthma by isocapnic hyperpnoea of cold air. *Eur Respir J* 10(10): 2243-2249, 1997.

38. Wesseling GJ, Vanderhoven-Augustin IM, Wouters EF. Forced oscillation technique in cold air provocation tests. *Thorax* 48(3): 254-259, 1993.

39. Heaton RW, Henderson AF, Gray BJ, Costello JF. The bronchial response to cold air challenge: evidence for different mechanisms in normal and asthmatic subjects. *Thorax* 38:506-511, 1983.

40. Wesseling GJ, Wouters EF. Respiratory impedance measurements in a dose-response study of isocapnic hyperventilation with cold air. *Respiration* 59(5): 259-264, 1992.

41. Bar-Yishay E, Ben-Dov I, Godfrey S. Refractory period after hyperventilation-induced asthma. *Am Rev Respir Dis* 127:572-574, 1983.

42. Ben-Dov I, Gur I, Bar-Yishay E, Godfrey S. Refractory period following induced asthma: contributions of exercise and isocapnic hyperventilation. *Thorax* 38:849-853, 1983.

43. Bleeker ER. Exercise-induced asthma. Physiologic and clinical considerations. *Clin Chest Med* 5:109-119, 1984.

44. Clausen J. Pulmonary Function Testing: Guidelines and Controversies. *Grune and Stratton, Inc.* Copyright 1984.

45. Cold air to detect bronchial hyper-reactivity. *Lancet* 1:1395-1396, 1980.

46. Forster RE II, Dubois A, Briscoe W, Fisher A. The Lung-Third Edition. *Year Book Medical Publishers, Inc.* Copyright 1986.

47. Haynes RL, Ingram RH, McFadden ER. An assessment of the pulmonary response to exercise in asthma and an analysis of the factors influencing it. *Am Rev Respir Dis* 114:739-742, 1976.

48. Heaton RW, Henderson AF, Costello JF. Cold air as a bronchial provocation technique. Reproducibility and comparison with histamine and methacholine inhalation. *Chest* 86:810-814,1984.

49. Lyons D, Marici FN. Cold Air Bronchial Challenge Testing. *The Pulmonary News. NSPT*. Spring 1993.

50. McFadden ER , Ingram RH Jr. Exercise-induced airway obstruction. *Annual Rev Physiol* 45:453-463, 1983.

51. Miller W, Scacci R, Gast L. Laboratory Evaluation of Pulmonary Function. *J.P. Lippincott Company*. Copyright 1987.

52. O'Byrne PM, Ryan G, Morris M, McCormack D, Jones NL, Morse JL, Hargreave FE. Asthma induced by cold air and its relation to nonspecific bronchial responsiveness to methacholine. *Am Rev Respir Dis* 125:281-285, 1982.

53. Strauss RH, McFadden ER, Ingram RH, Deal EC, Jaeger JJ. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. *J Clin Invest*. 61:433-440, 1978.

54. Tessier P, Ghezzo H, L'archeveque J, Cartier A, Malo J. Shape of the Dose-Response Curve to Cold Air Inhalation in Normal and Asthmatic Subjects. *Amer Rev Resp Dis* 136:1418-1423, 1987.

55. Zawadski DK, Lenner KA, McFadden ER. Comparison of Intraairway Temperatures in Normal and Asthmatic Subjects after Hyperpnea with Hot, Cold, and Ambient Air. *Amer Rev of Resp Dis* 138: 1553-1558, 1988.

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