DISSERTATION

DIETARY SODIUM CHLORIDE AS A MODERATOR OF THE SEVERITY OF EXERCISE-INDUCED ASTHMA

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY TIMOTHY DEREK MICKLEBOROUGH ENTITLED DIETARY SODIUM CHLORIDE AS A MODERATOR OF THE SEVERITY OF EXERCISE-INDUCED ASTHMA BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

DIETARY SODIUM CHOLRIDE AS A MODERATOR OF THE SEVERITY OF EXERCISE-INDUCED ASTHMA

The potential influence of diet on asthma and other forms of obstructive lung disease have received relatively little attention. Epidemiological evidence indicates an association between mortality from asthma and high dietary sodium chloride intake. Subsequent experiments have shown that bronchial reactivity is increased on high sodium chloride diets. Since 40 to 90% of asthmatics exhibit airway obstruction upon an exercise challenge, it has been suggested that subjects with exercise-induced asthma (EIA) will also be affected by alterations in dietary salt (NaCl). The working hypotheses were that: 1) a low salt diet (LSD) would improve and a high salt diet (HSD) would worsen post-exercise pulmonary function in subjects with EIA, and that changes in post-exercise pulmonary function maybe mediated by the sodium and/or chloride constituent of NaCl; 2) guinea pigs fed a HSD would exhibit increased airway obstruction compared to a NSD following a dry gas hyperpnea challenge, and that the airway response to hyperpnea is mediated by NaCl-induced leukotriene release.

Two sets of eight subjects with clinically diagnosed EIA and two sets of eight Control subjects volunteered for the first and second study. For the first study, subjects entered on their normal salt diet (NSD) and then were placed on either a LSD or a HSD

for two weeks. A one-week washout period occurred between diets before the subjects crossed over to the alternative diet for another two weeks. Twenty-four hour urine collections were obtained at the end of each treatment period. Subjects underwent treadmill testing to peak exercise, and pre- and post-exercise pulmonary function tests were performed. Changing dietary NaCl intake had no effect on pre-exercise pulmonary function in EIA and Control subjects, and had no effect on post-exercise pulmonary function in Control subjects. However, the LSD improved and the HSD worsened post-exercise pulmonary function in EIA subjects. At 15 minutes of recovery, forced expiratory volume in one-second (FEV_1) decreased $14 \pm 6\%$ on the LSD, $20 \pm 7\%$ on the NSD, and $24 \pm 6\%$ on the HSD in EIA subjects. In EIA subjects, tidal volume (V_T) and breathing frequency (f_B) selection varied during exercise with the NaCl diets, with higher V_T and lower f_B on the HSD, and the opposite on the LSD. This suggests greater airway obstruction on the HSD during exercise. The Control subjects demonstrated no changes in V_T or f_B selection on the different NaCl diets. Urinary sodium excretion was significantly higher on the HSD compared to the NSD and LSD in a graded fashion.

The subjects in the second study followed an identical testing protocol as the first study, except they followed either a LSD for two weeks (low sodium, low chloride) or a sodium bicarbonate ($NaHCO_3$; high sodium, low chloride) diet for two weeks, and then switched over to the alternative diet after a one-week washout period. Altering sodium or chloride had no effect on pre-exercise pulmonary function values in either group or post-exercise pulmonary function values in Control subjects. However, both the LSD and $NaHCO_3$ diet improved post-exercise pulmonary function in EIA subjects, compared to the NSD (which had a significantly higher chloride content compared to the other diets).

In EIA subjects, comparing pre- to post-exercise, FEV_1 decreased 7 ± 4% on the LSD, 14 ± 4% on the $NaHCO_3$ diet, and 19 ± 2% on the NSD. While the LSD diet did not normalize pulmonary function (compared to the Control subjects) it did improve it. Likewise, the $NaHCO_3$ diet also improved post-exercise pulmonary function in EIA subjects, but not to the extent of the LSD. This suggests that chloride may be a major contributor to this response. However, it appears that the presence of high sodium in the diet ($NaHCO_3$) prevents the total improvement seen with the LSD (low sodium, low chloride). Urinary excretion of sodium was significantly higher on the $NaHCO_3$ diet compared to the NSD and LSD in a graded fashion. In addition, urinary excretion of chloride was not significantly different between the $NaHCO_3$ diet and LSD, but was significantly higher for the NSD compared to the $NaHCO_3$ and LSD.

The third study involved using an animal model of EIA. Airway responses to dry gas hyperpnea ventilation challenge mimic EIA and have been used to model this condition in animals. The mechanisms of the airway response to hyperventilation have been extensively explored using guinea pigs. Thirty-two guinea pigs were used in this study. The animals were split into two groups. One group (n=16) followed a high salt (HS) diet (2% NaCl) for two weeks and the other group (n=16) followed a normal salt (NS) diet (0.75% NaCl) for two weeks. At the end of each treatment period the animals were cannulated for drug administration, tracheotomized and mechanically ventilated with a small animal ventilator. Airway responses to hyperpnea were measured as changes in airway pressures. The HS diet elicited significantly higher airway pressures compared to the NS diet following the first hyperpnea challenge, indicating greater airway obstruction on the HS diet. Prior to a second hyperpnea challenge, the animals

were either infused with Nordihydrouaiaretic acid (NDGA) which is a non-selective leukotriene (LT) biosynthesis and lipoxygenase (LO) inhibitor (BLO group), or saline (CON group). The LT-LO inhibitor resulted in significant blunting of the bronchoconstrictor response to the second hyperpnea challenge in the BLO groups, compared to the CON groups on both diets. However, the HS-BLO diet still elicited higher airway pressures than the NS-BLO diet during this post-hyperpnea period. One interpretation is that NaCl may mediate the release of LTs from effector cells in the airways in a dose dependent manner. Another interpretation is that NaCl loading enhances airway osmolarity. This increase in osmolarity provides an ideal environment for the release epithelial and mast-cell derived mediators and neuropeptides from sensory nerves. In addition, it is possible that NaCl loading exacerbates HIAO by increasing vascular volume in the bronchial circulation and hence microvascular pressure, leading to mucosal edema and narrowing of the airway lumen. This effect may work independent or in conjunction with the release of LTs in the development of HIAO.

In conclusion, this is the first study to demonstrate that the chloride constituent of NaCl may play a major role, along with sodium, in the severity of EIA, and that dietary NaCl loading may enhance the release of inflammatory mediators from cells in the airways of guinea pigs following dry gas hyperpnea.

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CHAPTER I

Introduction

The idea that diet may be important in asthma is long established, but both the precise formulation of the connection and its popularity have varied widely over time. Fifty years ago, it would have been a commonplace belief that nutrition was an important determinant of asthma. However, the highly restrictive diets that were imposed on patients with asthma, in the 1940s, were found to be more damaging than helpful, and consequently the interest in how diet may modify symptoms of asthma faded. Diet has been thought to influence asthma through the ingestion of allergens, mediators, mediator precursors (in particular some fatty acids), electrolytes, and antioxidants. Stoesser and Cook (106), in 1938, were the first to propose a possible connection between salt (NaCl) consumption and the severity of asthma. They observed that a low NaCl diet contributed to a decrease in symptoms in children with severe asthma.

Asthma morbidity and mortality are greater in communities adopting a more Western lifestyle, and in migrants as they move from rural underdeveloped to urban Westernized areas (25, 26, 113, 115). The lack of an adequate epidemiologic explanation for this phenomenon, coupled with the increase in NaCl consumption observed with urbanization, led Burney (25) to hypothesize that increased sodium intake might be in

part responsible for the increased morbidity and mortality of asthma. Burney tested the hypothesis using regional data from England and Wales and found a strong correlation between table NaCl purchases and mortality due to asthma in men and children of both sexes but not in women. Burney et al. (28) also found increased 24-hour urinary excretion of sodium, but not potassium, to be associated with increased airway responsiveness to histamine in a cross-sectional survey comprising 138 men, 18-64 years of age. The sample was enriched with subjects reporting wheeze. Several epidemiological studies since then have produced conflicting results, some showing a relation between asthma or bronchial reactivity and urinary sodium excretion or NaCl intake (92) and others showing no relation (14, 101).

To differentiate between cause and effect, intervention studies modifying consumption of NaCl and monitoring bronchial responsiveness and severity of asthma were necessary. In a small open study, doubling the intake of dietary NaCl for one month caused a modest increase in bronchial reactivity (60). A larger, randomized controlled trial demonstrated that bronchial response to histamine was greater in men on a low sodium diet (LSD) while they were taking slow sodium release capsules than when they were taking placebo (27). Two studies looked at air flow and asthmatic symptoms with conflicting results. An open trial of a normal, low, or high salt diet (HSD) in 17 subjects (nine men) found no relation between dietary consumption of NaCl and peak flow variability or use of beta-agonists (68). Carey et al. (31) studied 27 asthmatic men receiving 200 mmol of slow sodium release capsules or placebo daily in a random order, while ingesting a LSD. The LSD was associated with lower methacholine reactivity, consumption of bronchodilators, and symptom scores and higher peak flow

and forced expiratory volume in one second. In addition, Javaid et al. (60) studied the effect of changing dietary NaCl consumption on airway reactivity as determined by a histamine challenge test in asthmatic and non-asthmatic individuals. A high NaCl intake increased bronchial reactivity compared to the LSD in men and women. Changing dietary NaCl consumption had no effect on bronchial reactivity in the non-asthmatic subjects.

Since 40 to 90% of asthmatics experience airway narrowing following exercise challenge (61), it is reasonable to expect that altering NaCl consumption will influence post-exercise pulmonary function in subjects with exercise-induced asthma (EIA). To this end Mickleborough et al. (81) conducted a study assessing the effect of dietary NaCl loading and restriction on 15 clinically diagnosed subjects with EIA. They demonstrated that a HSD worsened and a LSD improved post-exercise pulmonary function volumes and flow rates as determined by spirometry.

At present, NaCl intake among adults and children in the United Kingdom and United States averages at least 9 g/day, with large numbers of adults consuming 12 g/day (45, 46), resulting in levels 10-15 times the basal sodium requirement for adults and growing children of 500 mg/day of sodium (45). Approximately 15% of current NaCl intake comes from NaCl added in cooking and at the table, while 10% comes from the natural content of foods. The remaining 75% of all NaCl eaten comes from NaCl added in processing and manufacturing of foods (45). A LSD in the United States is now feasible due to successful marketing of no-salt and low-salt products. There is increasing evidence that a high dietary NaCl intake may have a direct effect on strokes, independent of blood pressure; in the development of left ventricular hypertrophy;

progression of kidney disease; and increased urinary calcium excretion, which in the long term may be associated with bone demineralization. The eventual benefits of a modest reduction in NaCl intake may have implications far beyond those discussed in this dissertation.

Statement of the Problem

The primary goal of this research project was to determine the influence of high and low NaCl diets on post-exercise pulmonary function in subjects with clinically diagnosed EIA and to determine which ionic constituent of NaCl (sodium or chloride) was primarily responsible for these changes. A second goal was to determine whether altering NaCl consumption in guinea pigs mediates leukotriene-dependent hyperpnea-induced airway obstruction (HIAO).

Specific Aims

Exercise-induced asthma is a condition that affects 40 to 90% of asthmatics and 6 to 13% of the general non-asthmatic population, being more prevalent in children than in adults. Asthma is increasingly being recognized as a public health problem in the developing world. At present the pharmacological goals of treating EIA are aimed at preventing the onset of asthma episodes and treating breakthrough episodes that may occur following exercise. Asthma medication can become expensive and chronic use may result in severe bronchospasms and death. This clearly emphasizes the importance

of patient education and proper use of asthma medication. Because dietary NaCl restriction may reduce reliance on pharmacological agents, and represent a previously untried and potentially beneficial therapeutic intervention in EIA subjects with typical and high NaCl diets, this study had the following specific aims:

Study 1

1. Examine the effect of altering dietary NaCl consumption on ventilatory and metabolic variables during exercise in subjects with clinically diagnosed EIA and subjects with no medical history of EIA (Controls).
2. Examine the effect of altering dietary NaCl consumption on post-exercise pulmonary function in subjects with clinically diagnosed EIA and Control subjects.

Study 2

1. Determine the influence of high and low chloride diets on ventilatory and metabolic variables during exercise in subjects with clinically diagnosed EIA and Control subjects.
2. Determine the influence of high and low chloride diets on post-exercise pulmonary function in subjects with clinically diagnosed EIA and Control subjects.

Study 3

1. Examine the effect of changing NaCl consumption on HIAO in guinea pigs, as determined by changes in airway responses to a dry gas hyperpnea challenge.
2. Determine whether blocking leukotriene (LT) biosynthesis and the lipoxygenase (LO) pathway reduces the airway response to hyperpnea on different NaCl diets.

Hypotheses

The following hypotheses were tested ($p<0.05$):

Study 1

1. Increased dietary NaCl will worsen and decreased NaCl will improve post-exercise pulmonary function variables in subjects with EIA, but have no effect on post-exercise pulmonary function variables in Control subjects.
2. Increased dietary NaCl will increase tidal volume and decrease breathing frequency during exercise on the HSD compared to the LSD, with no changes being observed in the Control subjects.

Study 2

1. That both a low chloride, low sodium and a low chloride, high sodium diet will improve post-exercise pulmonary function in subjects with EIA

compared to a normal salt diet (NSD), but have no effect on post-exercise pulmonary function in Control subjects.

Study 3

1. Hyperpnea-induced airway obstruction will be exacerbated on a HSD compared to a NSD in guinea pigs, as exhibited by bronchoconstrictor responses to dry gas hyperpnea.
2. Blocking LT biosynthesis and the LO pathway will attenuate the airway response to hyperpnea on both diets, but the HSD will still demonstrate greater HIAO compared to the NS diet in guinea pigs.

CHAPTER II

LITERATURE REVIEW

Exercise-induced asthma (EIA) is a condition that is characterized by symptoms of cough, wheezing, and/or chest tightness after exercise (70, 76, 107, 119). EIA, exercise-induced airway narrowing, and exercise-induced bronchoconstriction are synonymous terms that describe a condition in which vigorous physical activity triggers acute airway narrowing with heightened airway reactivity (74). EIA is not an isolated disorder or a specific disease, but rather part of the asthmatic diathesis where exercise is one of the many stimuli that induces airflow limitation. Typically, maximal airflow obstruction occurs within 15 minutes after cessation of exercise with a subsequent slower spontaneous return to baseline airflows within 20 to 60 minutes (102).

Exercise, hyperventilation, and exposure to frigid air cause a transient increase in pulmonary resistance in approximately 75-80% of asthmatic subjects (47). For a fixed level of ventilation, colder, dryer inspired air exacerbates this response, whilst warmer more humidified air reduces its severity. Increasing the duration and strength of the stimulus also increases the magnitude of obstruction (47).

Epidemiology of EIA and asthma

EIA is a common condition that affects 40 to 90% of asthmatics (61) and 35 to40% of allergic non-asthmatics (114). Approximately, 6 to13% of the non-asthmatic general population suffers from EIA, being more prevalent in children than adults (30, 93). This wide range is spurious and derives from differences in the intensity of the exercise stress used to produce the phenomenon, variations in definition, a lack of uniformity in the methods used to detect the response, and failure to standardize the environmental variables that control the magnitude of the obstruction. In short, the true prevalence is unknown because the available data were not collected using appropriate standardized methodology. In experienced laboratories, however, virtually every asthmatic can be made to demonstrate a reduction in pulmonary mechanics to thermal provocations (i.e., exercise and hyperventilation) if the stimulus is intense enough.

The prevalence of asthma increased by approximately 50% from 33.4 per 1000 in 1982 to 50.7 per 1000 in 1991 based on data available from the United States National Center for Health Statistics, and on hospital discharge and annual health interview surveys for ages 0-45 years (99). The mortality rate from asthma increased by 40% between 1982 and 1991, from 13.4 per million population to 18.8 per million (103). In 1990, asthma cost an estimated $6.2 billion, with 10 million school days lost, 160,000 hospitalizations, 860,000 emergency room visits, and 1,254,000 visits to pediatricians. Understanding EIA may allow earlier detection of asthma and more timely treatment and prophylaxis, particularly since 29 to 51% of asthma is silent or undetected (83, 95), unless children are exercised.

Pathophysiology of EIA

Under most climatic conditions, the inspired air needs to be warmed and humidified to 37°C and 99.5% relative humidity before it reaches the alveoli. Breathing at rest usually occurs through the nose, which has a remarkable capacity to heat and humidify the inspired air. Even at moderately high flow rates, air inspired through the nose is fully heated and humidified by the time it reaches the oropharynx (71). However, during vigorous exercise the nose is bypassed, the frequency of breathing increases, and the ventilation rate increases 10- to 20-fold. Thus, the respiratory tract needs to condition much larger volumes of air over a much shorter time during exercise compared with rest.

Heat and water are added to the air from the respiratory mucosa as a direct function of the temperature and water vapor concentration gradients. During inspiration, the process of warming and humidifying the inspired air results in mucosal cooling. During expiration, as the air passes over the cooled mucosa, some heat is given back to the mucosa. As the temperature of the air decreases, its ability to hold water decreases, and hence some water condenses back to the airway surface. However, there is a net loss of water and heat from the airways as a result of conditioning of the inspired air. Theoretical predictions, using experimental data on intra-airway airstream temperatures (79), suggest that up to 40% of water may be provided by the lower airways during exercise, depending on the minute ventilation and inspired conditions (35, 36).

The primary source of the water for humidification in the lower airways is the airway surface liquid, and the source of its water is likely to be the bronchial circulation.

The airway surface liquid consists of two layers, the sol and the gel. The sol layer is a thin watery layer with a depth of 5 to 7 μm, approximately the same as the length of the cilia (97), and is often referred to as the periciliary fluid layer. The absolute volume of this fluid layer in the first 10 generations of airways is probably < 0.7 ml (5). The gel layer consists of mucus, which forms a discontinuous blanket (112) on top of the periciliary fluid layer. Water may also be provided by the mucus (glandular secretions) layer, as it contains 95% water (21).

The precise source of the airway surface liquid is unknown, and there are a number of possibilities. It is very likely that it is secreted by the epithelium and that this source is also responsible for the hydration of mucus (118). It may result from the liquefying effect of the cilia beating the mucus (118), although this is now thought unlikely. It may originate from the alveoli and move up by the mucociliary escalator, as suggested by Kiburn in 1968 (66). It may be produced by a difference in pH between the sol and gel layers, as at pH above 7.5, mucus becomes more liquid (49). The most likely source of the airway surface liquid, however, is the result of the effect of active ion transport (22, 121).

Whatever the source of the water, the airway surface fluid contains Na^+, Cl^-, K^+, and Ca^{2+} ions (63). Thus, there is considerable potential for water loss by evaporation to cause a significant increase in osmolarity of the airway surface liquid, if the rate of water loss exceeds the rate of water return during hyperpnea.

The mechanism unique to exercise that triggers EIA in sensitive subjects is unknown. Heat and water loss associated with an increase in minute ventilation during

exercise, along with rapid rewarming of the airways post-exercise, are believed to be causative. There are two popular hypotheses to explain the pathophysiology of EIA.

Thermal expenditure or respiratory heat exchange theory. McFadden et al. (78, 80) have suggested that EIA results from heat transfer from the bronchiolar blood vessels in the pulmonary vascular bed with heat loss during exercise; after exercise, the heat transfer is followed by rapid rewarming of these bronchiolar vessels, which causes dilatation and hyperemia of the vessels. The hyperemia is accompanied by an increase in permeability of the microvasculature and results in airway edema. Therefore, this theory is based on the vasculature of the airways. The bronchial vasculature consists of subepithelial capillaries that underlie the entire airway, from trachea to small bronchioles. Engorgement of these vessels could result in narrowed airways. It has been proposed that the airway narrowing of EIA is the end result of these vascular events and is independent of bronchial smooth muscle contraction or the release of inflammatory mediators (52, 75).

Water loss theory. Hyperventilation associated with strenuous exercise leads to water loss through the epithelium of the bronchial mucosa leading to a transient increase in airway osmolarity and changes in pH and temperature. Airway dehydration leading to hypertonicity of the airways provides an ideal environment for the release of mast cell-derived mediators, such as histamine, leukotrienes (LTs) and prostaglandins, and neuropeptides from sensory nerves (3, 9, 11) (Figure II-1). Bronchial smooth muscle contraction and airway narrowing are the results of these events (5, 6)

Unfortunately, there is no direct experimental evidence to support either the 'thermal" or the "osmotic" theories of EIA, and both remain hypotheses (Figure II-2).

However, the results of studies in animals suggest that increases in blood flow, vascular engorgement, airway wall thickening, mucosal edemas, or vascular leakage are unlikely to account for significant airway narrowing in the absence of smooth muscle contraction (20, 24, 89). Although the type of exercise and condition of inspired air during exercise are important in modifying the bronchoconstrictor response to exercise, the severity of the response is mainly determined by the duration and intensity of exercise. It is well established that the severity of EIA increases with increasing intensity and duration up to a plateau value (76). One of the physiological effects of exercise is to increase minute ventilation (V_E) in order to meet the increasing metabolic demand of exercising muscles. Deal et al. (39) demonstrated a unique relationship between the level of V_E achieved during exercise and the severity of the bronchoconstrictor response to exercise in asthma, and suggested that, for a given set of inspired air conditions, high V_E resulted in greater airflow obstruction than low levels of V_E.

Diagnosis and laboratory testing of EIA

EIA is clinically defined as a transient increase in post-exercise airway resistance, resulting in a greater than 10% fall in post-exercise forced expiratory volume in 1 second (FEV_1), compared to pre-exercise (44, 104). It has been suggested that a 15-20% post-exercise reduction in FEV_1 or peak expiratory flow rate (PEFR) is appropriate (38, 95). Further recommendations include decrements in forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$) ranging from 15-25% to be representative of EIA (1, 70).

The treadmill and cycle ergometer are the most commonly used testing devices in the laboratory for diagnosis of EIA. Many epidemiologists use free-range running

when screening large numbers of subjects. However, it is more difficult to monitor work intensity under free-running conditions. The type of activity chosen for exercise is not as important as ensuring that the subject is familiar with the equipment, can perform the testing safely, and can reach and maintain a level of work intensity associated with the production of EIA. There should be a means of monitoring heart rate during the exercise so that the work intensity can be regulated to meet the test requirements.

It is of paramount importance that the temperature and humidity of the air that is breathed by the exercising subject be documented. For research purposes these parameters need to be held constant from test to test and from individual to individual. In general, low humidity and cooler air temperature are more asthmogenic (76). Temperatures between 19 and 25°C with humidity $\leq$ 50% have been considered satisfactory (100, 104). Reproducible conditions can be easily produced by having the subject inhale compressed air at room temperature during the exercise (104).

Pulmonary function tests should be measured before exercise and at 1, 5, 10, and 15 minutes post-exercise (33, 44, 107, 114). For the treadmill test, EIA is typically diagnosed by employing a step stress protocol until 85-90% of predicted maximum heart rate (PMHR) is achieved, and then a further 5-8 minutes within this heart rate zone, keeping the treadmill speed and inclination constant (42-44). The subject's response to work should be monitored with changes in either ventilation or heart rate. Since continuous assessment of ventilatory effort is cumbersome and electrocardiographic monitoring is a necessary precaution, continuous evaluation of heart rate is the usual method (42-44).

It has been suggested that symptoms of EIA amongst athletes do not appear unless PMHR approaches 90-100% and the ambient air temperature is "cold" (94). With a cycle ergometer, EIA can readily be evoked in a susceptible individual with 4 minutes of work at 50-60% of the subject's predicted maximal oxygen consumption ($VO2_{max}$) (42-44, 94). With this load, ventilation reaches a level sufficiently high to cause a response in all but the most fit people. For those with a high level of fitness, exercise at 90% of $VO2_{max}$ may be required. In some elite athletes, it may be difficult to evoke symptoms in the laboratory because of the combination of activities and muscle groups used. This is particularly true for sports requiring high-intensity effort of variable duration. In such instances, these individuals should be tested in their usual environment while they perform the activities that induce their asthma (76).

Refractory Period

About 40 to 50% of subjects with EIA exhibit a refractory period, which is defined as the time period (usually about 1 hour) in which bronchoconstriction is not inducible with a secondary exercise challenge that follows a primary exercise stimulus. This tachypyylaxis (a reduced contractile response with repeated stimulation with the same concentration) applies only to subsequent exercise provocation and not to other stimuli such as an allergen challenge (18). The mechanism of this tachyphylactic response is not certain, but may involve altered responsivity of the bronchial circulation (51), mediator depletion (87), increases in catecholamines released during the first exercise challenge (16), and release of inhibitory prostaglandins (85).

Neural control of airway function

The airway smooth muscle cells and mucus producing cells are controlled by the autonomic nervous system and by chemical mediators in the extracellular environment, e.g., mediators that enter airway tissue via blood and mediators that are released locally by cells residing in airway tissue. Parasympathetic nerves via the vagus nerve innervate the airways and release acetylcholine and subsequently cause bronchoconstriction and mucus secretion when activated (10). Catecholamines that are released from sympathetic nerves, and catecholamines released from the adrenal medulla into the blood, may act on smooth muscle causing bronchodilation. There is also a third class of nervous system called nonadrenergic noncholinergic (NANC) nerves found in the airways of animals and humans (10). Excitatory NANC (e-NANC) nerves are readily demonstrated in rodent airways but there is still debate about their presence in human airways. NANC inhibitory (i-NANC) nerves release nitric oxide (NO) when activated (17), causing bronchodilation. The physiological effects of the NANC system can be demonstrated by administrating capsaicin to humans (58). Capsaicin has been shown to stimulate nonmyelinated C-fibers and causes airway smooth muscle contraction both by activating a vagal reflex or by the release of tachykinins.

Sensory nerves in animal and human airways contain several neuropeptides that have potent effects on target cells of the airways. These include the tachykinins substance P (SP) and neurokinin A (NKA). When activated, SP and NKA contract airway smooth muscle of several species, including humans (69). Damage to the airway epithelium, possibly due to airway thermal flux changes occurring after exercise in EIA subjects, may expose afferent nerve endings. These nerve endings may also be

stimulated by inflammatory mediators in the airway lumen. Bradykinin selectively stimulates C-fiber nerve endings and releases sensory neuropeptides from perfused guinea pig lungs (96).

Humoral control of airway function

Several locally generated chemical mediators alter airway tone and may affect the rate of fluid secretion or resabsorption. Histamine particularly is known to cause smooth muscle contraction, but it also increases capillary permeability, causing fluid exudation. The release of arachidonic acid, a 20-carbon polyunsaturated fatty acid (PUFA), from cell membrane phospholipds through the action of a family of phospholipases can result in the production of a wide variety of mediators that may be relevant in the pathogenesis of asthma (Figure II-3). These lipid mediators have traditionally been considered in two classes: mediators that result from the action of the enzyme cyclo-oxygenase on arachidonic acid, which are prostaglandins or thromboxane (prostanoids), and mediators that result from the action of the enzyme 5-lipoxygenase (5-LO) on arachidonic acid, which are the leukotrienes (LTs). These mediators are collectively known as eicosanoids. More recently, however, other products have been identified that result from the activity of different enzymes, such as 12- and 15-lipoxygenase.

The prostaglandins are most easily considered in two classes. There are stimulatory prostaglandins, such as PGD_2 and $PGF_{2\alpha}$, which are potent bronchoconstrictors (54, 109), and inhibitory prostaglandins, such as PGE_2, which can reduce bronchoconstrictor responses (116), and can attenuate the release of acetylcholine

from airway nerves (117). However, the differentiation of the prostaglandins into stimulatory and inhibitory classes is somewhat inappropriate. For example, both PGE_2 and $PGF_{2\alpha}$ can have different effects on the airways depending on the time after inhalation at which the response is measured (116). However, the main actions of PGE_2 and PGI_2 on airway function are to relax airway smooth muscle, to antagonize the contractile responses of other bronchoconstrictor agonists, and to inhibit the release of acetylcholine from airway cholinergic nerves.

The importance of LTs as mediators of airway inflammatory processes is increasingly being delineated. Arachidoinic acid is converted sequentially to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then to LT-A_4 by a catalytic complex consisting of 5-LO and the 5-LO-activating protein (FLAP). This interaction occurs at the perinuclear membrane where FLAP is localized. An increase in intracellular calcium enhances the affinity of FLAP for 5-LO (122). Subsequently, in the presence of LT-C_4 synthase, glutathione is added to LT-A_4 to yield LT-C_4, which is exported extracellularly, where the glutamic acid moiety is cleaved by gamma-glutamyltranspeptidase to form LT- D_4. Because they each contain cysteine, these molecules are known as cysteinyl-LTs (cys-LTs) or sulphidopeptide-LTs. All three cys-LTs have the same biological effects; however, LT-E_4 is much less potent than its precursors and is the excretory metabolite. Mast cells, eosinophils and alveolar macrophages are the cells in the lung that possess the enzymatic machinery to produce cysteinyl-LTs (41). The cys-LTs act on a single smooth muscle cell receptor in the airway, which has recently been designated cys-LT_1 receptor(8, 55, 62).

LT-C_4 and LT-D_4 can contract human airway smooth muscle *in vitro* and have greater than 1, 000 times more potency in this action than does histamine (34) and can increase airway mucus production (32) and induce microvascular leak. (64). LT-D_4 is a potent bronchoconstrictor, and interestingly has been implicated in the development of exercise refractoriness, via stimulation of inhibitory prostaglandin release (73). The cys-LTs have been detected after bronchospasm in the blood, BAL fluid, and urine of patients with asthma (86).

Evidence that eicosanoids play a role in mediating a portion of the bronchospam elicited by exercise derives from studies utilizing LT receptor antagonists and cyclooygenase inhibitors. The leukotriene D_4 antagonist MK-571 was shown to strongly inhibit exercise-induced bronchoconstriction in asthmatics (72). Similar effects were reported for the thromboxane synthase inhibitor OKY-046 (57), although its effects appear to be somewhat less pronounced. Prostaglandins may play a role in the refractoriness that occurs following exercise-induced bronchoconstriction because this phenomenon can be blocked by the cyclooxygenenase inhibitor flurbiprofen. This inhibitor also blocked tachyphylaxis to leukotriene D_4 (73). These results suggest that leukotriene D_4 may contribute to exercise-induced airway narrowing and at the same time stimulate the release of bronchodilator cyclooxygenase products such as PGE_2, which could attenuate further responses to bronchoconstriction.

Alterations in pulmonary function during exercise

There is evidence to suggest that airway obstruction occurs during exercise, as well as after exercise, in subjects with EIA. When exercise is prolonged for more than 15

minutes, bronchoconstriction may ensue during the exercise bout. Beck at al. (15) looked at constant load exercise on a cycle ergometer at 50% of maximal exercise capacity for 36 minutes, compared to an interval exercise with three cycles consisting of 6 minutes at 60% maximal capacity, alternating with 6 minutes at 40% maximal capacity. Interestingly, during the cycle of increasing and decreasing work, the FEV_1 tended to drop during the work of lesser severity and increase back to baseline during the greater level. The steady-state exercise showed a significant decrease in FEV_1 compared to baseline at 18, 24, and 30 minutes of exercise. The decreases in PEFR and FEV_1 were equivalent between the two protocols after the cessation of exercise.

Another study used more vigorous exercise at 60-85% of $VO2_{max}$ for 5 and 20 minutes (108). The FEV_1 rose at 2 minutes but fell significantly by 15 minutes of continuous exercise. This suggests that bronchoconstriction may occur during prolonged exercise and that it should not always be "run through". This should be contrasted with isocapnic hyperventilation, where no bronchoconstriction has been observed to occur during periods of hyperventilation lasting up to 16 minutes (19).

One could expect that a certain amount of mediator release would be triggered by airway drying or cooling during exercise. However, airway function for the most part is largely preserved during exercise (15), and most of the bronchoconstriction occurs after exercise (in most subjects). Two studies have shown that airways are less reactive to histamine when it is given during exercise or hyperventilation, consistent with some bronchodilator influence during periods of increased ventilation (59, 105). Thus, there may be one or more bronchoprotective mechanisms (increased sympathetic drive and inhibitory prostaglandins) operating during exercise.

Alterations in pulmonary function after exercise

Bronchodilation occurs during the first several minutes of exercise, presumably because of catecholamine release (4). The epinepherine is derived from the adrenal medulla, and the norepinepherine is derived from the sympathetic nervous system, which are activated during exercise. This may help to explain why EIA occurs maximally after and not during exercise. With EIA, pulmonary function classically declines 5-15 minutes after exercise has ceased, an event known as the early-phase response (90), with a gradual return to baseline values within 20-60 minutes (102). Some asthmatic subjects experience a second decline, known as the late-phase response, several hours after the early-phase response. Most studies demonstrate reductions in post-exercise pulmonary function in EIA subjects (30, 107). However, Gelb et al. (50) demonstrated that subjects with symptomatic asthma exhibited significant improvement in forced expiratory flow rates after 6 to 8 minutes of maximally tolerated exercise, indicating exercise-induced bronchodilation. Gelb et al. (50) assumed that the bronchodilator response to muscular effort may be "partly related to mechanical or other effects of hyperpnea and may be mediated in part by prostaglandins." Todaro (110) observed a similar response in highly trained asthmatic athletes. The post-exercise bronchodilation observed in these studies can possibly be attributed to an increase in catecholamines, either binding to an increased number of beta-2 receptors or a greater receptor sensitivity, as a result of increased muscular effort. It is apparent that given the same work load, the intensity of post-exercise bronchoconstriction is lessened through training (53).

Treatment of EIA

Potential nonpharmacological intervention. Airway hyperresponsiveness is believed to exist intrinsically or to develop in response to airway inflammation resulting from exposure to allergens, respiratory infections, cigarette smoke, or other irritants. Acting as antioxidants or through influences on immune function, vitamins C and E and beta carotene in the diet may reduce airway inflammation, thereby decreasing the severity of asthma or preventing altogether the expression of asthma in susceptible individuals (82, 111).

The fatty acid composition of the diet – in particular, the relative amounts of n-6 and n-3 PUFAs – may also be associated with the risk of asthma (23, 91, 111). Increased production of prostaglandins and LTs, active in bronchoconstriction and neutrophil chemotaxis, occurs when linoleic acid is converted to arachidonic acid (111). Intake of linoleic acid may enhance the production of the arachidonic acid metabolites PGD_2, $PGF_{2\alpha}$, LTB_4, LTC_4, and LTD_4, which can promote airway inflammation and subsequently bronchoconstriction. In contrast, omega-3 fatty acids compete with arachidonic acid to form less active prostanoids and LTs, thereby potentially acting to reduce airway inflammation and bronchoconstriction (111). Further studies are needed to clarify the role of omega-3 fatty acids and their potential therapeutic role in asthma.

It has been demonstrated that high dietary magnesium loads may eleviate symptoms of asthma. Okayama et al. (88) showed that 20 mEq of magnesium infused over 20 minutes increased FEV_1 from 62% of predicted to 80% during a 'mild' asthma attack. Dominguez et al. (40) demonstrated in erythrocytes taken from asthmatic patients that intracellular magnesium levels are inversely and strongly related to

bronchial reactivity and suggests that magnesium alterations may play a role in the pathogenesis of asthma bronchoconstriction by unknown mechanisms.

Epidemiological studies show that there is an increased prevalence of asthma in communities adopting a Western lifestyle, while a low prevalence is found in some of the world's poorest areas (113). Regional mortality data from England and Wales showed a strong correlation between asthma mortality and regional per person purchases of salt (25). Several interventional studies have been performed (27, 29, 31, 60) that have demonstrated that restriction of salt intake in asthmatic subjects can reduce bronchial reactivity.

Occasionally a vigorous warm-up period of 30-60 minutes can effectively create submaximal EIA, and most importantly, induce a 2-4 hour refractory period in which further bouts of exercise will not create further bronchospam. The mechanism for this induction is unclear but may relate to depletion of mediators from mast cells so that further reactivity cannot occur during subsequent provocation until mediators are replenished (37)

Pharmacological intervention. The preferred treatment before exercise is an inhaled fast acting beta-agonist taken 15 minutes before the exercise bout. If this is not adequate, then cromolyn sodium or nedocromil can be added to this pretreatment; both are mast cell stabilizers. A long acting beta agonist inhalant, such as salmeterol, can be give a number of hours before exercise. This drug has been shown to prevent EIA in subjects for up to 10-12 hours (65).

The chronic daily medications for EIA include cromolyn sodium, nedocromil, inhaled long acting beta-agonists (bronchodilators), or inhaled corticosteroids. Inhaled

corticosteroids (56) are not effective for pretreatment just before exercise, but they may provide benefit if used daily on a chronic basis. They are most effective in preventing airway obstruction before exercise in subjects who have underlying asthma.

The recently introduced antileukotriene agents include LT receptor antagonists (e.g. Montelukast and Zafirlukast) and 5-LO inhibitors (e.g. Zileuton). The anitleukotrienes are currently recommended for long term asthma therapy but have been shown to block the bronchial response in exercise challenge studies(67).

Other agents that may be used in combination with beta-agonists and mast cell stabilizers include anticholinergic agents, theophylline, calcium channel blockers, antihistamines, alpha-agonists and oral beta-agonists.

Heparin has been given by inhalation to evaluate the pathophysiology of EIA and has been shown to significantly block EIA (48). This may be the result of a direct blocking effect on mast cells rather than a smooth muscle relaxing effect. In addition, indomethacin (98) and inhaled furosemide (84) also have been shown to protect against EIA. These medications are currently used as research tools and are not used in pharmacotherapy.

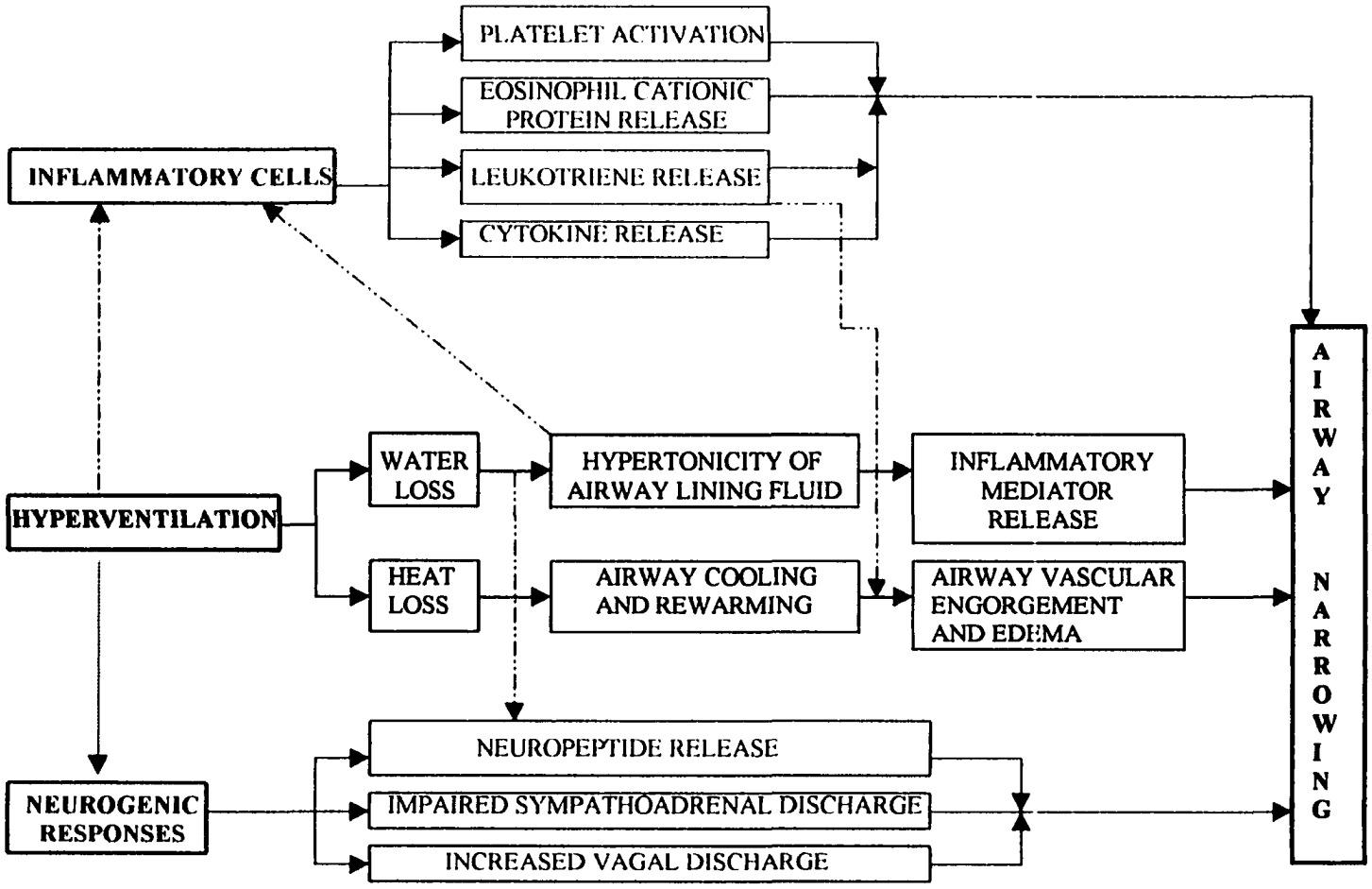

Figure II-1. Pathophysiological changes during exercise in asthma implicated as a cause of airway narrowing.

(Adapted from Makker and Holgate, 1994).

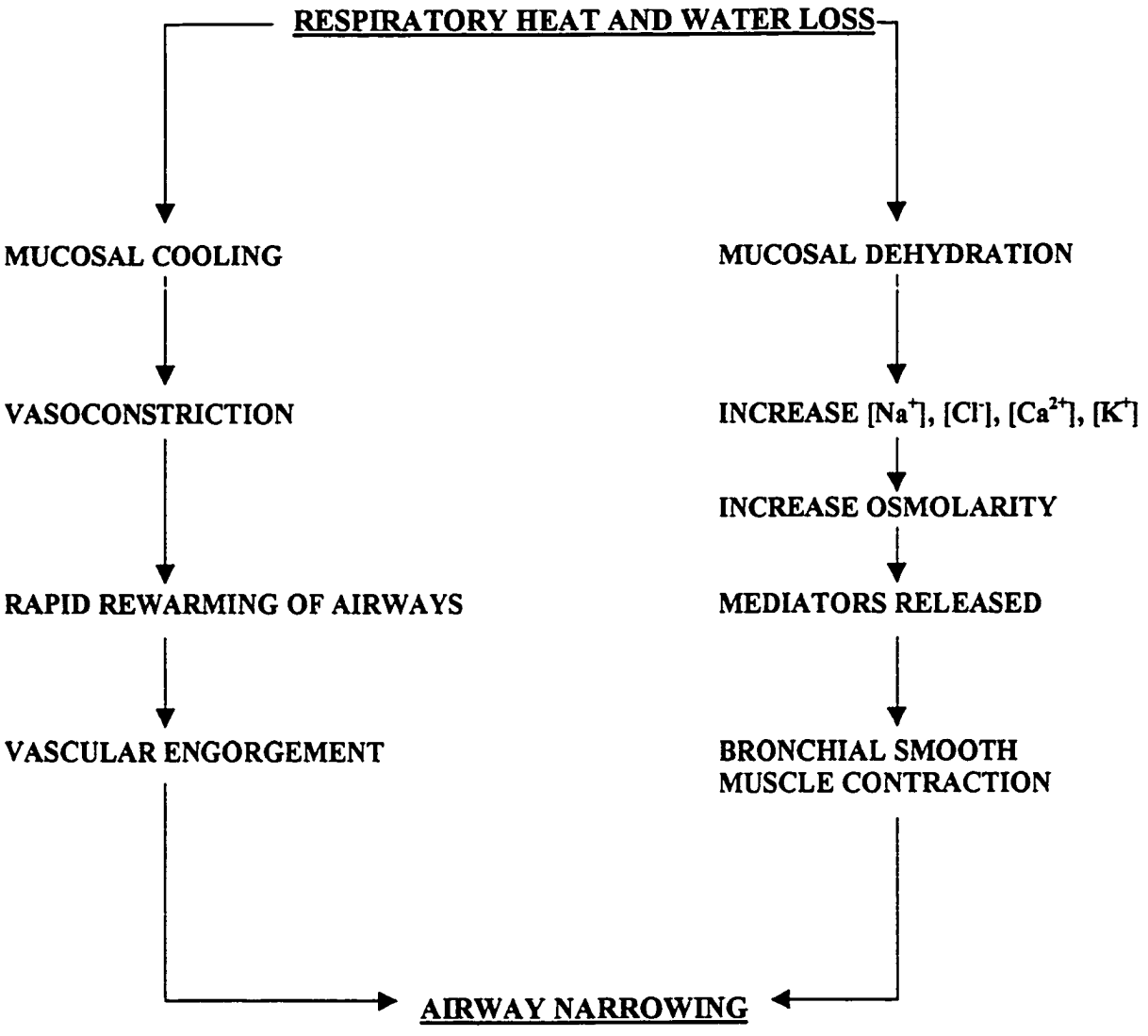

Figure II-2. There are two hypotheses with respect to how water loss causes the airways to narrow. One suggests from airway cooling (McFadden, 1990; Gilbert et al. 1992), and the other, mucosal dehydration and an increase in osmolarity (Anderson, 1984; Anderson et al. 1982).

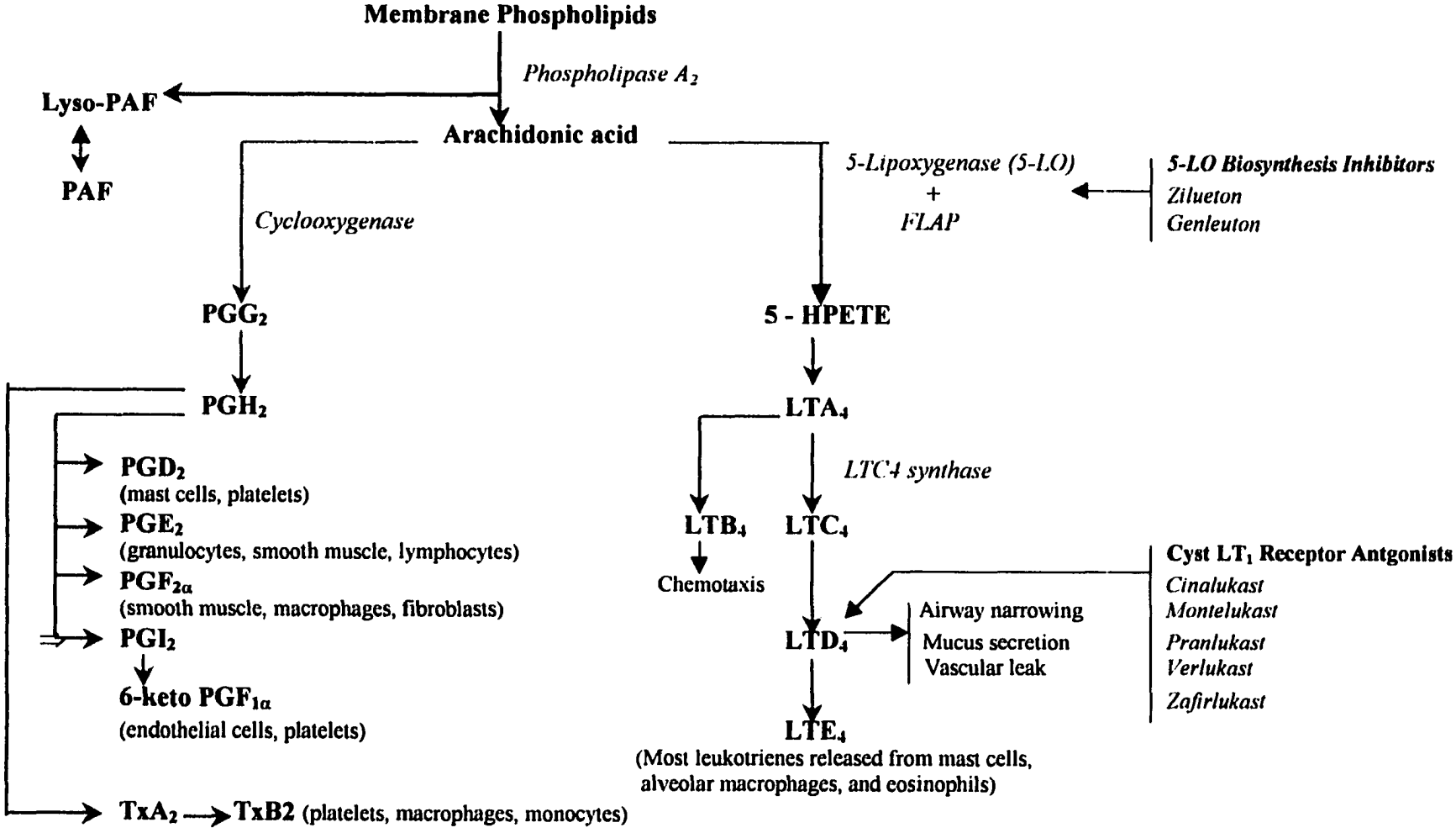

Figure II-3. The arachidonic acid cascade, showing synthesis of prostaglandins via the cyclooxygenase pathway and leukotrienes via the 5-lipooxygenase pathway (adapted from O'Bryne, 1997).

CHAPTER III

Dietary salt alters pulmonary function in exercise-induced asthmatics

Abstract

Purpose: Exercise-induced asthma (EIA) occurs in approximately 90% of asthmatics and is defined as a transient increase in post-exercise airway resistance. The mechanism responsible has not been delineated. Epidemiological studies have implicated dietary salt (NaCl) as being responsible for changes in bronchial reactivity in asthmatics. Therefore, the purpose of this study was to determine if manipulation of dietary NaCl could influence pulmonary function in EIA subjects. **Methods:** Eight subjects with clinically diagnosed EIA and eight subjects without EIA (Control) participated in a double-blind crossover study. Subjects entered the study on a normal salt diet (NSD), and then were placed either on a low salt diet (LSD) or a high salt diet (HSD) for two weeks. A one-week washout period occurred between diets before subjects crossed over to the alternative diet. Twenty-four hour urine collections confirmed compliance to the diets. Pre- and post-exercise pulmonary function tests were performed at the end of each treatment period. Subjects underwent treadmill testing to peak exercise. During exercise, ventilatory and metabolic variables were measured by indirect calorimetry. **Results:** Changing dietary NaCl intake had no effect

on pre-exercise pulmonary function in either group. Dietary NaCl had no effect on post- exercise pulmonary function in Control subjects. However, the LSD improved and the HSD worsened post-exercise pulmonary function in EIA subjects. In EIA subjects, compared to pre-exercise values, post-exercise forced expiratory volume in 1-second (FEV_1) decreased only 14 $\pm$ 6% on the LSD, decreased by 20 $\pm$ 7% on the NSD, and decreased further by 24 $\pm$ 6% on the HSD at 15-min post-exercise. Similar patterns were observed for forced vital capacity, forced expiratory flow rate at 25-75% FVC and peak expiratory flow rates. In EIA subjects, tidal volume (V_T) and breathing frequency (f_B) selection during exercise varied with the NaCl diets, with higher V_T and lower f_B on the HSD, and the opposite for the LSD. This suggests greater airway resistance during the HSD. Manipulation of salt diets had no effect on V_T or f_B in Control subjects. Ventilation-perfusion mismatching was enhanced with the HSD in EIA subjects, as demonstrated by an increased V_D/V_T ratio. **Conclusion**: While a LSD did not normalize post-exercise pulmonary function in EIA subjects, it did improve it. Salt is likely a modifier of the EIA response and not the cause of EIA. In conclusion, a HSD caused an increase in the severity of EIA and a LSD represents a potential therapeutic intervention for EIA subjects.

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Introduction

Epidemiological studies have linked dietary salt (NaCl) to the prevalence and severity of asthma (8-11). In general, the higher the NaCl intake within a population, the greater the prevalence of asthma and the greater the severity of asthma (8-11). Additionally, most (12, 13, 15, 28, 47, 51, 55) but not all (7, 18, 20, 39), interventional studies have implicated dietary NaCl and transmembrane sodium ion transport with the regulation of airway smooth muscle tone, and suggests that a diet high in NaCl may increase the severity of asthmatic symptoms and bronchial reactivity (28, 55).

While the mechanism by which dietary NaCl may lead to airway reactivity changes is not known, it is possible that the sodium ion influences smooth muscle contractility, including bronchial and vascular smooth muscle (15, 55). The influence of dietary NaCl on circulating blood volume and, consequently, on hemodynamics and pulmonary function can not be ruled out as another possibility.

The influence of dietary NaCl on EIA has been previously investigated. Mickleborough et al (45) demonstrated that NaCl loading worsened and NaCl restriction improved pulmonary function at 5 minutes post-exercise in 15 clinically diagnosed EIA subjects. The influence of changes in dietary NaCl on post-exercise pulmonary function in Control (non-asthmatic) subjects was not evaluated. If dietary NaCl enhances vascular and bronchial reactivity, then it is reasonable to expect that EIA would be worsened by elevated dietary NaCl and improved by dietary NaCl restriction. Therefore, this investigation was performed to determine if alterations in dietary NaCl would influence the severity of EIA. The experimental hypothesis tested in the current study was that increased dietary NaCl would worsen and decreased dietary NaCl

would improve pulmonary function variables at 1, 5, 10, and 15 minutes post exercise in subjects with clinically diagnosed EIA, but have no effect on pulmonary function in Control (non-EIA) subjects.

Methods

Subjects. Eight, clinically-diagnosed EIA subjects and eight subjects with no history or signs of EIA (Control) volunteered for this study. The subjects were recruited from a university student population, and each subject completed a health questionnaire and gave written informed consent to participate prior to enrollment in the study. The study was approved by the Human Subjects Committee Review Board at Colorado State University. All EIA subjects had a history of post-exercise shortness of breath, and intermittent wheezing, relieved by bronchodilator therapy after exercise; but were otherwise free of atopic asthma. All EIA subjects had been using prescribed medication prior to participation in the study. All EIA subjects tested positive for EIA, as indicated by a drop of greater than 10% in post-exercise FEV_1 compared to pre-exercise values (22), while none of the Control subjects tested positive for EIA. Blood pressure measurements, using a sphygomanometer, were taken at the beginning of the study to screen for hypertension, and on the first day and every third day of the treatment period in order to check for any abnormal rises in blood pressure. Blood pressure was also measured pre- and post-exercise

Study Design. The study was conducted as a double-blind randomized crossover trial over five consecutive weeks, with a one-week washout period between each two-week treatment period. All subjects entered the study on a normal salt diet (NSD),

which varied according to each subject's regular dietary NaCl intake; after which they were randomly assigned to either a low salt diet (LSD) or high salt diet (HSD) for two weeks. Thereafter, they followed a one-week washout (NSD) and then switched to the alternative diet for the remaining two weeks. A base diet was provided by means of a menu plan and required all subjects, whether on the LSD or HSD, to consume approximately 1500 mg/day of sodium and ~ 2250 mg/day of chloride. During the HSD period, the base diet was supplemented with 10 one-gram salt capsules per day, which equaled 4000 mg/day of sodium and ~ 6,000 mg/day of chloride . However, for the LSD, the base diet was supplemented with an equivalent dose of sucrose, placebo capsules.

Protocol. Each subject was instructed to avoid any strenuous physical activity 24 hours prior to the exercise test and to refrain from using therapeutic drugs prior to the exercise test; time from last dose being dependent on the type of drug being used. At an initial screening test and at the end of each treatment period, the subjects underwent exercise challenge testing, used for the diagnosis of EIA (17, 21-23). The subjects were required to perform pre-exercise pulmonary function tests. All subjects were required to have pre-exercise FEV_1 values that were at least 80% of baseline values, achieved during initial screening, to ensure that the subjects' values were not depressed prior to exercise (30). An exercise test on a treadmill followed in order to induce a bronchospasm. Pulmonary function tests were conducted at 1, 5, 10 and 15 minutes post-exercise. After all post-exercise pulmonary function tests were completed, the subjects were allowed the use of their bronchodilators. Pulmonary function tests were repeated 5 minutes after bronchodilator therapy to ensure lung function had returned to near pre-exercise test

values and to confirm that the decrement in flow rates was due to bronchospasm. Pulmonary function tests were conducted on each subject using a Sensormedics Vmax AutoBox D_L (Sensormedics Corporation, Yorba Linda, CA). Subjects were required to perform a total of three acceptable spirograms, of which two measurements of FEV_1 with a maximal difference of 100ml were obtained with the larger value used in data analysis (1).

The exercise stress test protocol required each subject to run on a Quinton Treadmill (Model 640, Series 90, Quinton Instrument Company, WA) using a standard graded protocol of incrementally increasing workloads up to ~ 85-90% of predicted maximum heart rate (PMHR) (17, 21-23). Once the target heart rate was achieved a constant load protocol was applied, which required each subject to exercise at a steady state for a further 6 minutes at the target heart rate. This protocol differed in treadmill speed and inclination for each subject in order to achieve the heart rate criteria. However, the same workload over the same period of time was performed by each subject on each study day, and speed/elevation were matched. Heart rate was determined from the ECG and monitored continuously (Quinton 4500 Stress Test Monitor, Quinton Instruments, Seattle, WA). At the end of the 6 minute steady-state exercise, the treadmill was elevated by 1% per min until volitional fatigue in order to achieve a measure of peak exercise. Environmental conditions were 23°C and 50% relative humidity. During the exercise, breath-by-breath analysis of expired gases was accomplished by open circuit spirometry (SensorMedics 2900 Metabolic Cart, Sensormedics Corporation, Yorba Linda, CA).

Collection of 24-Hour Urine Samples. Twenty-four hour urine excretion of electrolytes was measured at the beginning of the study and at the end of each treatment to monitor dietary sodium compliance. Each subject voided urine into 2500 ml bottles, which were collected on one of the last three days of each treatment period. The volume was recorded, and sodium and potassium concentrations were measured on a Beckman Astra analyzer (Beckman Instruments Inc., La Brea, CA) using ion specific electrodes. Urinary creatinine concentration was determined by a modified Jaffe rate reaction, using the same instrument, in order to verify the completeness of the 24-hour urine samples.

Statistical Analysis. Data were analyzed using the SigmaStat 2.03 statistical package and SYSTAT 8.0 (SPSS Inc., Chicago, IL). Pre-exercise and post-exercise pulmonary function values, and metabolic and ventilatory data were examined for the effect of diet (LSD, NSD, HSD) and the presence of EIA by a 2-factor repeated measures ANOVA, with both treatment and time as "within-subject" effects. A Tukey's post-hoc multiple pairwise comparison was used to isolate the differences. When the F-ratio was significant, an omega squared (ω^2) was used to assess the magnitude of the treatment effect (i.e., what percent variance is accounted for by the treatments). Power was calculated at 0.925, using a sample size of n = 8 (per group). The effect of a treatment administered in one period may continue to be present at the start of the following period. This condition is referred to as carry-over effect. To lessen the chances of a significant carry-over effect in the current study, a wash-out period was included between treatments to allow the effect of a treatment given in one period to be "washed-out" of each subject's system before beginning a different treatment in the next period.

However, even when a wash-out period is included, significant carry-over effects may remain. Therefore, the data were analyzed for the presence of carry-over effects between treatments by an ANOVA for a 2 x 2 crossover design (31). All statistical tests of significance were set at $p < 0.05$. Data are expressed as mean $\pm$ SEM.

Results

Subject characteristics are shown in Table III-1, which demonstrates that the two groups, EIA and Control, were well-matched according to age, physical characteristics and fitness level. Table III-2 indicates that the subjects were using traditional short-acting or "rescue" medications, and none were on long term "maintenance" medications. All subjects completed the study and no EIA subjects were dropped from the study due to failure to test positive for EIA at the initial screening. Subjects demonstrated dietary compliance with sodium enhancement on the HSD and sodium restriction on the LSD as indicated by the 24-hr urinary excretion of sodium (Table III-3). Neither potassium nor creatinine excretions were altered by the diets (Table III-3).

Baseline (pre-exercise) pulmonary function values are presented in Table III-4. Baseline pulmonary function was not altered by the diets in either group. All baseline pulmonary function values for the three diets and for each group fell within the normal parameters established for males and females at rest (16), indicating that no airflow limitations were present.

Post-exercise pulmonary function values are presented in Table III-5 and III-6. For Control subjects (Table III-5) there were no significant differences in post-exercise pulmonary function values by time or diet. However, the EIA subjects (Table III-6)

demonstrated significant reductions, in post-exercise pulmonary function on all diets at all post-exercise times. For FVC, FEV_1, $FEF_{25-75\%}$ and PEFR, the post-exercise values were highest on the LSD, less on the NSD, and less still on the HSD. The FEV_1/FVC ratio decreased post-exercise on all diets; however, the FEV_1/FVC ratio was not altered between diets.

The differential effect of dietary salt on the percent change in FEV_1 pre- to-post exercise for Control and EIA subjects is shown in Figure III-1 and Figure III-5 respectively. Control subjects demonstrated no significant difference ($p>0.05$) in the percent change in FEV_1, FVC (Table III-2), $FEF_{25-75\%}$ (Table III-3) and PEFR (Table III-4) pre- to post-exercise on any diet. EIA subjects showed a significant reduction ($p<0.05$) in percent change in FEV_1, FVC (Table III-6), $FEF_{25-75\%}$ (Table III-7) and PEFR (Table III-8) pre- to post-exercise, in a gradation of response from LSD to NSD to HSD. However, the minimum 10% reduction in post-exercise FEV_1, used for the diagnosis of EIA, was observed during all diets.

Changes in pulmonary function at pre-exercise and 1, 5, 10 and 15 minutes post-exercise for Control subjects are shown in Figures III-9 to III-12, and Figures III-13 to III-16 for EIA subjects. The Control subjects demonstrated no significant differences by time for each of the diet periods. The EIA subjects demonstrated significant reductions in all the pulmonary function variables measured by time for each of the diet periods.

An ω^2 was performed on pulmonary function values for EIA subjects at 15 minutes post-exercise and indicated that 80% (FEV_1), 92% (FVC), 82% ($FEF_{25-75\%}$) and 76% (PEFR) of the variance was accounted for by the treatments. In addition, an ω^2 was

performed on ventilatory variables at peak exercise and indicated that 70% (V_T), 72% (f_b) and 77% (V_D/V_T) was accounted for by the treatments.

Ventilatory and metabolic variables measured during exercise for EIA and Control subjects are presented in Tables III-7 to III-16. Control subjects (Tables III-7-III-11) demonstrated no significant differences in any of the variables measured during exercise by diet. The EIA subjects, however, showed significant differences in tidal volume, V_T (Table III-13), which increased with exercise intensity for all diets, but was significantly higher for the HSD and significantly lower for the NSD and LSD, in a graded response at peak exercise. Respiratory rate, f_b (Table III-13) likewise increased with exercise intensity for all diets. However, in contrast to the results for V_T, fb was significantly higher for the LSD and significantly lower for the HSD, with the NSD being intermediate, at peak exercise.

The ratio of physiologic dead space to tidal volume, V_D/V_T (Table III-15) decreased with exercise intensity on both the LSD and NSD in EIA subjects. The LSD resulted in lower values for V_D/V_T throughout the exercise bout, while the HSD increased this ratio, which then remained elevated during all levels of exercise.

There were some differences noted in the minute ventilations (V_E) at peak exercise, while on different diets, however, these were not significant (NS). Because ventilation plays a role in triggering EIA symptoms (2, 3, 40), the effect of ventilation on the pre- to post-exercise change in FEV_1 was examined by regression analysis (Figure III-17 and III-18). It is apparent that NS variations in maximal ventilation achieved during exercise did not correlate with decrements in pulmonary function observed on the three diets in EIA and Control subjects. Therefore, post-exercise pulmonary function changes

are a function of diet and not changes in minute ventilation occurring at peak exercise. Control subjects elicited the same dissociation between V_E and changes in pre- to post-exercise change in FEV_1.

The results of the 2 x 2 ANOVA crossover design indicated that carry-over effects were not significant ($p>0.05$) for all measures of lung function in EIA subjects. Table III-17 details the results of an ANOVA 2 x 2 crossover design for FEV_1 at 10 minutes post-exercise. The ANOVA table clearly shows that carry-over effects were not significant ($p>0.05$). Furthermore, there were no significant period effects.

Figures III-25 to III-28 provide visual confirmation of the statistical tests in Table III-17. Figures III-25 and III-26 are plots for each group showing the change in each subject's response over the two treatment periods. Group one started the LSD in period one and then crossed over to the HSD in period two, while Group two started on the HSD in period one and then crossed over to the LSD in period two. It is clear from these graphs that FEV_1 decreased on the HSD and improved on the LSD in both groups. Figure III-27 indicates that the direct treatment difference is the same in both periods, i.e., there is no group-by-period interaction. The test for parallelism is the t-test (or F-test) for carry-over effects. If this is not significant (as is the case in the current study), then the lines are considered parallel, except for random perturbations. Real data will contain random variation and so exactly parallel lines will not be seen, even if there is no interaction. Figure III-28 is a plot of the sum of the observations versus the difference between the observations for each subject in each group. The convex hulls of the subjects in each group are also shown in each of these plots. Significant overlap of the groups in the horizontal (total axis) indicates no carry-over effect, while separation in

the horizontal indicates a carry-over effect. Comparing the groups in the horizontal plane clearly demonstrates a good deal of overlap and hence there is strong evidence to reject the hypothesis that carry-over effects are significant. Separation of the two groups in the vertical plane (difference axis) demonstrates that the results induced by the two treatments are significantly different. In the present study, the convex hulls of the two groups have no overlap, indicating significant differences between the HSD and LSD; as were shown in Table III-17. Similar results were observed in EIA subjects for FEV_1 at 1, 5, and 15 minutes post exercise and for the other three lung functions measured (FVC, $FEF_{25-75\%}$ and PEFR) at 1, 5, 10, and 15-minutes post-exercise. Control subjects elicited a similar response, in that carry-over effects were not significant for all measures of lung function at 1, 5, 10, and 15-minutes post-exercise.

Discussion

The current study represents the first report of altered post-exercise pulmonary function in EIA subjects, compared to Control subjects, as a result of dietary changes in NaCl consumption. Reductions in dietary NaCl improved post-exercise pulmonary function and elevated dietary NaCl worsened post-exercise pulmonary function in EIA subjects. Restricting dietary NaCl did not, however, normalize post-exercise pulmonary function in these EIA subjects. In general, there was a graded improvement in post-exercise pulmonary function as EIA subjects changed from the HSD to the NSD to the LSD. There were no changes in post-exercise pulmonary function for the Control subjects on the different diets.

Dietary goals were met and compliance was successful as sodium excretion fell significantly while on the LSD and rose significantly while on the HSD. This occurred without a change in potassium excretion or glomerular filtration rate (creatinine excretion). Thus, a graded dose of dietary sodium was achieved in this study from 1, 335 mg/day - 1, 580 mg/day (LSD) to 6, 750 mg/day - 10, 547 mg/day (HSD).

In the current study, the FVC maneuver provided an indirect measure of the flow resistive properties of the lung. Pre- to post-exercise changes were evaluated in both groups. All expiratory lung volumes and flow rates for the EIA subjects improved in a dose-response manner from HSD to LSD, suggesting less airway obstruction. No differences were demonstrated for these values with respect to the different diets for Control subjects.

The exercise protocol used in this study was a typical clinical protocol used to induce and diagnose EIA (56). The subjects exercised for 6 min at 85-90% PMHR (steady state exercise), after which they continued on until volitional fatigue (peak exercise). Therefore, the exercise protocol was of reasonably short duration, but reached high intensity.

The pattern of exercise ventilation during exercise differed with diet in the EIA subjects, even though there were no differences in exercise minute ventilation between diets. The HSD resulted in a higher V_T and lower f_b selection, while the LSD reversed this pattern, lower V_T and higher f_b. This breathing pattern suggests increased airways resistance with the HSD and decreased airway resistance with the LSD. The pattern of breathing chosen by the EIA subjects on the HSD suggests increased airway resistance during exercise. Typically, subjects with elevations in airway resistance will increase V_T

and decrease f_b in order to reduce the work of breathing. The reversal of this pattern elicited by the LSD may indicate a reduction in airway resistance by the same reasoning. However, no direct measure of airway resistance was performed during exercise in the present study. Other studies have reported a similar ventilatory pattern during exercise as demonstrated in the present study in subjects with EIA versus those subjects without EIA (Control) or with successful treatment (6, 25).

Previous studies have shown the presence of ventilation-perfusion mismatching post-exercise in EIA subjects (57, 62). In the current study, the elevation of V_D/V_T with the HSD suggests reduced ventilation relative to perfusion and improved V_D/V_T or improved ventilation-perfusion matching with the LSD. However, these data must be interpreted cautiously, since the pattern of ventilation to perfusion by this method is difficult to interpret in the face of changing total ventilation and cardiac output induced by exercise (62).

The present study confirms earlier work (3-5, 29, 41, 53, 54) that have demonstrated increased resistance to airflow in EIA subjects during the later stages of an exercise bout. Beck et al. (5) reported a decline in FEV_1 during exercise of varying intensity in asthmatic subjects with documented EIA compared to non-asthmatic subjects. Johnson et al. (29) also compared asthmatics with documented EIA to non-EIA subjects during exercise. The EIA subjects demonstrated an elevation in end-expiratory lung volume and airflow limitations compared to the non-EIA subjects. Beck et al. (4) compared subjects with EIA versus non-asthmatic subjects. The purpose of the study was to compare maximal flows from FVC maneuvers to measures of pulmonary resistance, such as using an esophageal balloon and measuring flow and pressure

changes. The asthmatic subjects demonstrated increased airways resistance during exercise, during both techniques. Suman at al. (54) compared the changes in airway function during exercise and voluntary hyperventilation, while measuring pulmonary responses to both modes of hyperpnea in the same asthmatic subjects, while attempting to match minute ventilation. A greater airway resistance during was observed during the later stages of the exercise bout than during the isocapnic hyperventilation, even though both modes of challenge produced similar degrees in the post-hyperpnic period.

The mechanism by which dietary NaCl may influence EIA is unknown. Since the mechanism of EIA itself has not been determined, it would be speculative to suggest a possible mechanism for the interaction of dietary NaCl with EIA. Changes in dietary sodium intake, and its influence on the post-exercise flow rates at 5 minutes, in 15 subjects with EIA was conducted recently by Mickleborough et al. (45). They demonstrated that two weeks of NaCl loading worsened and two weeks of NaCl restriction improved post-exercise pulmonary function in EIA subjects. Data have also been published on the possible relationship between asthma and dietary NaCl intake, and have been mainly epidemiological with limited experimental evidence. As early as 1938, Stoesser and Cook (52) reported that a LSD contributed to a decrease in symptoms in children with severe asthma. Burney (8, 10, 11) conducted epidemiological studies in England and Wales, and a strong correlation was noted between table salt purchases and asthma mortality in both men and children. Experimental studies have concentrated on the effect of manipulating dietary sodium intake on airway responsiveness. A small study demonstrated a significant increase in airway responsiveness to histamine in male and female asthmatics on a HSD (28). A

randomized double-blind crossover challenge designed to test the effect on airway responsiveness to histamine in asthmatic subjects on a LSD while taking a NaCl supplement or a placebo demonstrated an increase in airway responsiveness in those receiving the sodium supplementation. In addition, a significant association between bronchial reactivity and 24-hour sodium excretion was observed in males but not female asthmatics (12). A double blind, placebo-controlled crossover design study demonstrated that a change from a HSD to a LSD resulted in a significant reduction in airway responsiveness to methacholine ($FEV_{1.0}$) and PEF (15). A more recent study (55) carried out an investigation to study dietary sodium intake and airway response to methacholine in relation to cellular sodium transport in asthmatics. The results suggested that a serum-borne factor found in asthmatic serum caused an increased permeability of cell membranes, thereby stimulating sodium influx into cells (which is related to the degree of hyper-responsiveness), independent of the effect of dietary sodium loading on airway responsiveness. Other studies have failed to find an association between sodium intake and asthma (or its surrogate, airways responsiveness) (7, 19, 20, 39) and therefore the evidence for an association between dietary sodium and asthma remains controversial.

It is unclear how variations in dietary sodium may lead to airway reactivity changes. However, sodium transport has been implicated in many aspects of the regulation of airway smooth muscle tone (15, 50, 55). A high sodium intake has been shown to inhibit Na^+/K^+ ATPase in erythrocytes of normotensive males (59). Enhanced dietary sodium loading expands blood volume and may trigger the release of endogenous ouabain (26, 59), that inhibits Na^+/K^+ ATPase. The resulting inhibition of

the Na^+/K^+ ATPase would be expected to increase levels of intracellular sodium and, in turn, to increase calcium via inhibition of Na^+/Ca^{2+} exchange (46), as well as, enhancing release of inflammatory mediators (14, 24, 44). Increased airway smooth muscle tone with pump inhibition is supported by animal experiments (50), but has not been shown in studies with humans. The pathological events involved in asthma, such as the release of inflammatory mediators, microvascular leakage, and mucous secretion are also calcium dependent. Therefore, any defect in the control of intracellular calcium can account not only for increased airway responsiveness, but also increased secretory responses. The mechanisms responsible for increased bronchial reactivity may be due directly or indirectly to hormonal or chemical changes associated with increased sodium loads, or to changes in the physical properties of cell membranes.

It has been shown that airway mucosal edema can have a profound effect upon airway function (27) in EIA. An increased blood volume in the bronchial circulation caused by dietary NaCl loading could exert an important influence on airway diameter. An increase in vascular volume and microvascular pressure might have substantial effects on airway function in the face of mediator-induced increased vascular permeability leading to a thickening of the mucosa (edema), thereby narrowing airway diameters; possibly amplifying the effects of increased smooth muscle tone (42). It is also possible that dietary NaCl loading may increase airway osmolarity leading to airway drying and an exacerbation of EIA symptoms.

The focus of previous studies has been directed at elucidating a possible mechanism whereby the cation, sodium influences bronchial reactivity in asthmatics. However, it is possible that the anion component of dietary NaCl, chloride, plays a

significant role in the severity of asthma and EIA. Medici et al. (43) investigated the effects of NaCl loading and restriction in 14 asthmatics. They substituted sodium chloride with sodium citrate in an equimolar concentration and demonstrated that while bronchial reactivity in these subjects was sensitive to changes in dietary NaCl, it was most likely mediated by the sodium ion, and not the chloride ion. However, in hypertension research, there have been several reports that implicate the chloride ion as the main contributor to elevated blood pressures during NaCl loading (32-38, 48, 49, 58, 60, 61).

In conclusion, this study has shown that elevated dietary NaCl worsened and dietary NaCl restriction consumption improved post-exercise pulmonary function in EIA subjects. It should be noted that a restriction in dietary NaCl did not normalize post-exercise pulmonary function in the EIA subjects. It is likely that elevations in dietary NaCl consumption does not cause EIA, but rather enhances the underlying mechanism and effects of exercise on pulmonary function in subjects with EIA. It is possible that dietary NaCl restriction will work in the opposite manner. Whether it is the sodium or chloride ion in NaCl that is responsible for changes in post-exercise pulmonary function in EIA subjects has yet to be determined.

References

1. Standardization of spirometry--1994 update. Statement of the American Thoracic Society. *Am J Respir Crit Care Med* 152: 1107-1136, 1995.

2. Anderson, S. D., and E. Daviskas. The airway microvasculature and exercise induced asthma. *Thorax* 47: 748-52, 1992.

3. Beck, K. C. Control of airway function during and after exercise in asthmatics. *Med Sci Sports Exerc* 31: S4-11, 1999.

4. Beck, K. C., R. E. Hyatt, P. Mpougas, and P. D. Scanlon. Evaluation of pulmonary resistance and maximal expiratory flow measurements during exercise in humans. *J Appl Physiol* 86: 1388-95, 1999.

5. Beck, K. C., K. P. Offord, and P. D. Scanlon. Bronchoconstriction occurring during exercise in asthmatic subjects. *Am J Respir Crit Care Med* 149: 352-7, 1994.

6. Bevegard, S., B. O. Eriksson, V. Graff-Lonnevig, S. Kraepelien, and B. Saltin. Respiratory function, cardiovascular dimensions and work capacity in boys with bronchial asthma. *Acta Paediatr Scand* 65: 289-96, 1976.

7. Britton, J., I. Pavord, K. Richards, A. Knox, A. Wisniewski, S. Weiss, and A. Tattersfield. Dietary sodium intake and the risk of airway hyperreactivity in a random adult population. *Thorax* 49: 875-80, 1994.

8. Burney, P. A diet rich in sodium may potentiate asthma. Epidemiologic evidence for a new hypothesis. *Chest* 91: 143S-148S, 1987.

9. Burney, P. The origins of obstructive airways disease. A role for diet? [editorial; comment]. *Am J Respir Crit Care Med* 151: 1292-3, 1995.

10. Burney, P. G. The causes of asthma--does salt potentiate bronchial activity? Discussion paper. *J R Soc Med* 80: 364-7, 1987.

11. Burney, P. G., J. R. Britton, S. Chinn, A. E. Tattersfield, A. O. Papacosta, M. C. Kelson, F. Anderson, and D. R. Corfield. Descriptive epidemiology of bronchial reactivity in an adult population: results from a community study. *Thorax* 42: 38-44, 1987.

12. Burney, P. G., J. E. Neild, C. H. Twort, S. Chinn, T. D. Jones, W. D. Mitchell, C. Bateman, and I. R. Cameron. Effect of changing dietary sodium on the airway response to histamine. *Thorax* 44: 36-41, 1989.

13. Burney, P. G. J., J. R. Britton, S. Chinn, A. E. Tattersfield, H. S. Platt, A. O. Papcosta, and M. C. Kelson. Response to inhaled histamine and 24 hour sodium excretion. *Br Med J* 292: 1483-1486, 1986.

14. Cabado, A. G., M. R. Vieytes, and L. M. Botana. Inhibition of Na+/K+ ATPase under hypertonic conditions in rat mast cells. *Life Sci* 63: 1227-37, 1998.

15. Carey, O. J., C. Locke, and J. B. Cookson. Effect of alterations of dietary sodium on the severity of asthma in men. *Thorax* 48: 714-8, 1993.

16. Crapo, R. O., A. H. Morris, and R. M. Gardner. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 123: 659-64, 1981.

17. Cropp, G. J. The exercise bronchoprovocation test: standardization of procedures and evaluation of response. *J Allergy Clin Immunol* 64: 627-33, 1979.

18. Demissie, K., and P. Ernst. Is increased dietary salt intake a cause of increased airway responsiveness or a marker of an unhealthy life style? [editorial]. *Respir Med* 88: 79-81, 1994.

19. Demissie, K., P. Ernst, K. Gray Donald, and L. Joseph. Usual dietary salt intake and asthma in children: a case-control study. *Thorax* 51: 59-63, 1996.

20. Devereux, G., J. R. Beach, C. Bromly, A. J. Avery, S. M. Ayatollahi, S. M. Williams, S. C. Stenton, S. J. Bourke, and D. J. Hendrick. Effect of dietary sodium on airways responsiveness and its importance in the epidemiology of asthma: an evaluation in three areas of northern England. *Thorax* 50: 941-7, 1995.

21. Eggleston, P. A. Laboratory evaluation of exercise-induced asthma: methodologic considerations. *J Allergy Clin Immunol* 64: 604-8, 1979.

22. Eggleston, P. A. Methods of exercise challenge. *J Allergy Clin Immunol* 73: 666-9, 1984.

23. Eggleston, P. A., R. R. Rosenthal, S. A. Anderson, R. Anderton, C. W. Bierman, E. R. Bleecker, H. Chai, G. J. Cropp, J. D. Johnson, P. Konig, J. Morse, L. J. Smith, R. J. Summers, and J. J. Trautlein. Guidelines for the methodology of exercise challenge testing of asthmatics. Study Group on Exercise Challenge, Bronchoprovocation Committee, American Academy of Allergy. *J Allergy Clin Immunol* 64: 642-5, 1979.

24. Franzius, D., M. Hoth, and R. Penner. Non-specific effects of calcium entry antagonists in mast cells. *Pflugers Arch* 428: 433-8, 1994.

25. Freeman, W., M. G. Nute, S. Brooks, and C. Williams. Responses of asthmatic and non-asthmatic athletes to prolonged treadmill running. *Br J Sports Med* 24: 183-90, 1990.

26. Haddy, F. J., and M. B. Pamnani. Role of dietary salt in hypertension. *J Am Coll Nutr* 14: 428-38, 1995.

27. Hogg, J. C., P. D. Pare, and R. Moreno. The effect of submucosal edema on airways resistance. *Am Rev Respir Dis* 135: S54-6, 1987.

28. Javaid, A., M. J. Cushley, and M. F. Bone. Effect of dietary salt on bronchial reactivity to histamine in asthma. *Br Med J* 297: 454, 1988.

29. Johnson, B. D., P. D. Scanlon, and K. C. Beck. Regulation of ventilatory capacity during exercise in asthmatics. *J Appl Physiol* 79: 892-901, 1995.

30. Johnson, J. D. Statistical considerations in studies of exercise-induced bronchospasm. *J Allergy Clin Immunol* 64: 634-41, 1979.

31. Jones, B., and M. G. Kenward. The 2 x 2 cross-over trial with continuous data. In: *Design and Analysis of Cross-over trials*. London: Chapman and Hall, 1989, p. 16-88.

32. Julian, B. A., J. H. Galla, G. P. Guthrie, Jr., and T. A. Kotchen. Renin and aldosterone responses to short-term NaCl or NaHCO3 loading in man. *J Lab Clin Med* 100: 261-8, 1982.

33. Kotchen, T. A., J. H. Galla, and R. G. Luke. Contribution of chloride to the inhibition of plasma renin by sodium chloride in the rat. *Kidney Int* 13: 201-7, 1978.

34. Kotchen, T. A., R. G. Luke, C. E. Ott, J. H. Galla, and S. Whitescarver. Effect of chloride on renin and blood pressure responses to sodium chloride. *Ann Intern Med* 98: 817-22, 1983.

35. Kurtz, T. W. Dietary chloride as a possible determinant of NaCl - sensitive hypertension (HTN) in man. *Kidney International* 31: A301, 1987.

36. Kurtz, T. W., H. A. Al-Bander, and R. C. Morris, Jr. "Salt-sensitive" essential hypertension in men. Is the sodium ion alone important? *N Engl J Med* 317: 1043-8, 1987.

37. Kurtz, T. W., and R. C. Morris. Dietary chloride and bicarbonate as determinants of desoxycortocosterone hypertension. *J Hypertens* 2: 371-373, 1984.

38. Kurtz, T. W., and R. C. Morris, Jr. Dietary chloride as a determinant of "sodium-dependent" hypertension. *Science* 222: 1139-41, 1983.

39. Lieberman, D., and D. Heimer. Effect of dietary sodium on the severity of bronchial asthma [see comments]. *Thorax* 47: 360-2, 1992.

40. Mahler, D. A. Exercise-induced asthma. *Med Sci Sports Exerc* 25: 554-61, 1993.

41. Mahler, D. A., K. Faryniarz, T. Lentine, J. Ward, E. M. Olmstead, and G. T. O'Connor. Measurement of breathlessness during exercise in asthmatics. Predictor variables, reliability, and responsiveness. *Am Rev Respir Dis* 144: 39-44, 1991.

42. McFadden, E. R., and I. A. Gilbert. Exercise-induced asthma as a vascular phenomenon. In: *Exercise-Induced Asthma*, edited by E. R. McFadden. New York: Marcel Dekker, Inc, 1999, p. 115-135.

43. Medici, T. C., A. Z. Schmid, M. Hacki, and W. Vetter. Are asthmatics salt-sensitive? A preliminary controlled study. *Chest* 104: 1138-43, 1993.

44. Meyer, G., S. Doppierio, P. Vallin, and L. Daffonchio. Effect of frusemide on Cl-channel in rat peritoneal mast cells. *Eur Respir J* 9: 2461-7, 1996.

45. Mickleborough, T. D., L. Cordain, R. W. Gotshall, and A. Tucker. A low sodium diet improves indices of pulmonary function in exercise-induced asthma. *J Exer Physiol* 3: 46-54, 2000.

46. Pavord, I. D., S. Dutt, O. J. Carey, D. Spence, S. Brand, and R. F. Bing. Red blood cell sodium transport in asthma. *Thorax* 45: 799, 1990.

47. Pistelli, R., F. Forastiere, G. M. Corbo, V. Dell'Orco, G. Brancato, N. Agabiti, A. Pizzabiocca, and C. A. Perucci. Respiratory symptoms and bronchial responsiveness are related to dietary salt intake and urinary potassium excretion in male children. *Eur Respir J* 6: 517-22, 1993.

48. Sharma, A. M., C. Cetto, U. Schorr, K. P. Spies, and A. Distler. Renal acid-base excretion in normotensive salt-sensitive humans. *Hypertension* 22: 884-90, 1993.

49. Shore, A. C., N. D. Markandu, and G. A. MacGregor. A randomized crossover study to compare the blood pressure response to sodium loading with and without chloride in patients with essential hypertension [published erratum appears in J Hypertens 1988 Nov;6(11):i]. *J Hypertens* 6: 613-7, 1988.

50. Souhrada, M., and J. F. Souhrada. Immunologically induced alterations of airway smooth muscle cell membrane. *Science* 225: 723-725, 1984.

51. Sparrow, D., G. T. O'Connor, B. Rosner, and S. T. Weiss. Methacholine airway responsiveness and 24-hour urine excretion of sodium and potassium. The Normative Aging Study. *Am Rev Respir Dis* 144: 722-5, 1991.

52. Stoesser, A., and M. Cook. Possible relation between electrolyte balance and bronchial asthma. *Am J Dis Child* 56: 943-44, 1938.

53. Suman, O. E., M. A. Babcock, D. F. Pegelow, N. N. Jarjour, and W. G. Reddan. Airway obstruction during exercise in asthma. *Am J Respir Crit Care Med* 152: 24-31, 1995.

54. Suman, O. E., K. C. Beck, M. A. Babcock, D. F. Pegelow, and A. W. Reddan. Airway obstruction during exercise and isocapnic hyperventilation in asthmatic subjects. *J Appl Physiol* 87: 1107-13, 1999.

55. Tribe, R. M., J. R. Barton, L. Poston, and P. G. Burney. Dietary sodium intake, airway responsiveness, and cellular sodium transport. *Am J Respir Crit Care Med* 149: 1426-33, 1994.

56. Virant, F. S. Exercise-induced bronchospasm: epidemiology, pathophysiology, and therapy. *Med Sci Sports Exerc* 24: 851-5, 1992.

57. Wagner, P. D., G. Hedenstierna, and G. Bylin. Ventilation-perfusion inequality in chronic asthma. *Am Rev Respir Dis* 136: 605-12, 1987.

58. Weinberger, M. H. Sodium chloride and blood pressure [editorial]. *N Engl J Med* 317: 1084-6, 1987.

59. Weissberg, P. L., M. J. West, M. J. Kendall, M. Ingram, and K. L. Woods. Effect of changes in dietary sodium and potassium on blood pressure and cellular electrolyte handling in young normotensive subjects. *J Hypertens* 3: 475-80, 1985.

60. Whitescarver, S. A., C. E. Ott, B. A. Jackson, G. P. Guthrie, and T. A. Kotchen. Salt-sensitive hypertension: contribution of chloride. *Science* 223: 1430-32, 1984.

61. Wyss, J. M., M. Liumsiricharoen, W. Sripairojthikoon, D. Brown, R. Gist, and S. Oparil. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension* 9: III171-5, 1987.

62. Young, I. H., P. Corte, and R. E. Schoeffel. Pattern and time course of ventilation-perfusion inequality in exercise- induced asthma. *Am Rev Respir Dis* 125: 304-11, 1982.

Table III-1. Subject characteristics.

Group	Gender M/F	Age (years)	Weight (kg)	Height (cm)	$VO2_{peak}$ (NSD) (ml/min/kg)
EIA	4,4	23 ± 1.7	66 ± 3	170 ± 3	43.6 ± 2.8
Control	1,7	23 ± 1.0	60 ± 4	166 ± 2	44.1 ± 5.8

Values are means ± SEM. There are no significant differences for any variables between diets ($p>0.05$).

Table III-2. Subject medications for EIA subjects.

Subject	Medication
1	Terbutaline (Bricanyl)
2	Albuterol (Ventolin)
3	Albuterol (Ventolin)
4	Terbutaline (Bricanyl)
5	Albuterol (Ventolin)
6	Albuterol (Ventolin)
7	Terbutaline (Bricanyl)
8	Albuterol (Ventolin)

Medications are all short-acting β_2 agonists used for rescue medication. No subjects appeared to be on drugs used for maintenance therapy.

Table III-3. Twenty-four hour urinary excretion of sodium, potassium, and creatinine (mg/day).

Group	LSD	NSD	HSD
EIA:			
Sodium	1,335 ± 227[a]	2,414 ± 416[b]	6,750 ± 1,159[c]
Potassium	1,802 ± 325	2,362 ± 652	2,973 ± 1,410
Creatinine	1,305 ± 136	1,353 ± 102	1,311 ± 99
Sodium normalized to creatinine	1.02 ± 0.32[a]	1.78 ± 0.38[a,b]	5.14 ± 0.70[c]
24-hour volume (ml)	1,434 ± 225[a]	1,761 ± 230[b]	2,351 ± 228[c]
Control:			
Sodium	1,580 ± 173[a]	3,478 ±425[b]	10,547 ± 1,868[c]
Potassium	2,561 ± 417	2,405 ± 435	2,213 ± 252
Creatinine	1,174 ± 253	1,485 ± 218	1,820 ± 204
Sodium normalized to creatinine	1.34 ± 0.81[a]	2.34 ± 0.60[a,b]	5.79 ± 1.50[c]
24-hour volume (ml)	2,065 ± 159[a]	2,537 ± 187[b]	3,187 ± 306[c]

Values are mean ± SEM. Letters [a,b,c] designate significance ($p<0.05$) among values, when letters are different. There are no significant differences among potassium or creatinine values ($p>0.05$).

Table III-4. Baseline pulmonary function.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
EIA:					
LSD	4.13 ± 0.31	3.37 ± 0.21	82.0 ± 2	3.44 ± 0.22	6.10 ± 0.66
NSD	4.09 ± 0.30	3.33 ± 0.18	81.0 ± 2	3.53 ± 0.27	6.14 ± 0.63
HSD	3.92 ± 0.36	3.18 ± 0.25	81.0 ± 3	3.35 ± 0.30	5.89 ± 0.74
Control:					
LSD	4.63 ± 0.30	3.87 ± 0.24	84.0 ± 3	4.08 ± 0.45	8.37 ± 0.80
NSD	4.70 ± 0.26	3.92 ± 0.23	83.0 ± 2	4.27 ± 0.43	7.61 ± 0.81
HSD	4.62 ± 0.26	3.71 ± 0.27	80.0 ± 2	4.03 ± 0.43	8.80 ± 0.70

Values are means ± SEM. There are no significant differences for any variables among diets or between groups ($p>0.05$). FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-sec; $FEF_{25-75\%}$, forced expiratory flow rate from 25-75% of FVC; PEFR, peak expiratory flow rate. LSD, low salt diet; NSD, normal salt diet; HSD, high salt diet.

Table III-5. Pulmonary function post-exercise in Control subjects.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
LSD:					
1min	4.56 ± 0.30	3.90 ± 0.25	86.0 ± 3	4.51 ± 0.51	8.41 ± 0.71
5min	4.62 ± 0.33	3.94 ± 0.27	83.0 ± 3	4.42 ± 0.55	8.50 ± 0.71
10min	4.60 ± 0.33	3.97 ± 0.28	86.0 ± 3	4.32 ± 0.54	8.46 ± 0.72
15min	4.73 ± 0.33	3.99 ± 0.28	84.0 ± 3	4.07 ± 0.34	8.59 ± 0.74
NSD:					
1min	4.55 ± 0.28	3.86 ± 0.22	85.0 ± 3	4.52 ± 0.46	7.93 ± 0.78
5min	4.64 ± 0.28	3.90 ± 0.21	84.0 ± 3	4.40 ± 0.45	8.01 ± 0.77
10min	4.65 ± 0.28	3.89 ± 0.23	84.0 ± 2	4.29 ± 0.43	8.14 ± 0.78
15min	4.71 ± 0.30	3.95 ± 0.26	84.0 ± 2	4.31 ± 0.49	7.79 ± 0.74
HSD:					
1min	4.54 ± 0.28	3.80 ± 0.25	84.0 ± 3	4.21 ± 0.60	8.58 ± 0.68
5min	4.52 ± 0.29	3.78 ± 0.25	84.0 ± 3	4.05 ± 0.48	8.38 ± 0.74
10min	4.58 ± 0.29	3.70 ± 0.29	81.0 ± 4	4.10 ± 0.44	8.55 ± 0.76
15min	4.56 ± 0.30	3.68 ± 0.29	81.0 ± 4	4.14 ± 0.44	8.44 ± 0.79

Values are means ± SEM. There were no significant differences in Control subjects for post-exercise values by time or diet ($p>0.05$).

Table III-6. Pulmonary function post-exercise in EIA subjects.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
LSD:					
1min	3.86 ± 0.35*a	3.14 ± 0.27*a	82.0 ± 3a	3.30 ± 0.32*a	5.62 ± 0.72*a
5min	3.86 ± 0.38*a	3.03 ± 0.29*a	80.0 ± 3a	3.08 ± 0.32*a	5.49 ± 0.74*a
10min	3.92 ± 0.38*a	2.99 ± 0.28*a	76.0 ± 3*a	2.92 ± 0.33*a	5.26 ± 0.78*a
15min	3.88 ± 0.38*a	2.90 ± 0.31*a	75.0 ± 2*a	2.80 ± 0.36*a	5.17 ± 0.82*a
NSD:					
1min	3.72 ± 0.37*a	2.99 ± 0.30*a,b	81.0 ± 3a	3.05 ± 0.36*b	5.39 ± 0.78*a,b
5min	3.71 ± 0.36*c	2.76 ± 0.29*c	74.0 ± 3*b	2.48 ± 0.37*b	4.94 ± 0.81*b
10min	3.72 ± 0.37*c	2.76 ± 0.29*c	74.0 ± 3*a	2.58 ± 0.32*b	4.90 ± 0.81*c
15min	3.63 ± 0.38*c	2.65 ± 0.33*c	72.0 ± 3*b	2.39 ± 0.38*b	4.68 ± 0.83*b
HSD:					
1min	3.51 ± 0.39*b	2.85 ± 0.29*b	82.0 ± 3a	2.91 ± 0.40*b,c	5.22 ± 0.77*b
5min	3.38 ± 0.42*b	2.57 ± 0.32*b	77.0 ± 4*a	2.37 ± 0.40*b,c	4.79 ± 0.80*b
10min	3.31 ± 0.43*b	2.51 ± 0.31*b	77.0 ± 3*a	2.29 ± 0.37*c	4.52 ± 0.83*b
15min	3.19 ± 0.41*b	2.42 ± 0.33*b	76.0 ± 4*a	2.15 ± 0.40*c	4.40 ± 0.84*b

Values are means ± SEM. *$p<0.05$ compared to respective pre-exercise value. Letters a,b,c refer to comparisons by diet for the post-exercise time period within specific variable; different letters designate significant difference ($p<0.05$). Values with the same letter are not statistically significant ($p>0.05$).

Table III-7. Cardiovascular changes occurring during exercise in Control subjects.

	LSD	NSD	HSD
HR (bpm)			
Rest	73 ± 3.1	87 ± 3.7	70 ± 2.8
Steady state exercise	148 ± 9.7	157 ± 3.9	159 ± 5.5
Peak Exercise	176 ± 7.3	171 ± 4.3	173 ± 6.5
HR % predicted max (bpm)			
Rest	41 ± 2.0	45 ± 1.9	42 ± 1.6
Steady state exercise	85 ± 1.9	88 ± 2.0	86 ± 2.7
Peak exercise	98 ± 3.6	95 ± 2.4	96 ± 3.1
O_2 Pulse (ml/beat)			
Rest	3.7 ± 0.6	3.4 ± 0.4	4.3 ± 0.3
Steady state exercise	17.7 ± 1.3	15.2 ± 1.1	17.5 ± 1.1
Peak Exercise	19.4 ± 1.3	17.1 ± 1.5	19.1 ± 1.7

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p \geq 0.05$. HR, heart rate; HR%, HR as a percentage of maximum heart rate (220-Age); O_2 Pulse, oxygen pulse (VO_2/HR).

Table III-8. Breathing pattern and exercise ventilation changes occurring during exercise in Control subjects.

	LSD	NSD	HSD
V_T (L)			
Rest	0.80 ± 0.10	0.87 ± 0.10	0.73 ± 0.23
Steady state exercise	2.2 ± 0.16	2.4 ± 0.18	2.3 ± 0.19
Peak Exercise	2.3 ± 0.21	2.4 ± 0.39	2.3 ± 0.23
f_b (breaths/min)			
Rest	13.6 ± 3.0	14.8 ± 2.4	15.0 ± 3.0
Steady state exercise	30.6 ± 2.5	26.8 ± 2.3	31.4 ± 3.0
Peak exercise	50.0 ±3.3	45.5 ± 2.1	46.1 ± 1.8
$V_{E\ BTPS}$ (L/min)			
Rest	10.9 ± 0.9	12.9 ± 1.4	10.9 ± 1.2
Steady state exercise	67.3 ± 3.8	64.3 ± 5.5	72.3 ± 3.5
Peak exercise	115 ± 10.2	109 ± 5.3	106 ± 11.6

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p \geq 0.05$. V_T, tidal volume; f_b, respiratory frequency; $V_{E\ BTPS}$, minute ventilation.

Table III-9. Oxygen uptake and carbon dioxide output changes occurring during exercise in Control subjects.

	LSD	NSD	HSD
VCO_{2STPD} (L/min)			
Rest	0.20 ± 0.01	0.26 ± 0.06	0.24 ± 0.03
Steady state exercise	2.10 ± 0.16	2.16 ± 0.22	2.23 ± 0.14
Peak Exercise	3.18 ± 0.22	2.72 ± 0.20	3.01 ± 0.34
VO_{2STPD} (L/min)			
Rest	0.27 ± 0.03	0.30 ± 0.05	0.30 ± 0.05
Steady state exercise	2.62 ± 0.17	2.39 ± 0.28	2.78 ± 0.18
Peak Exercise	3.42 ± 0.26	2.92 ± 0.36	3.30 ± 0.42
VO_{2STPD} (ml/min/kg)			
Rest	4.1 ± 0.3	4.2 ± 0.8	4.7 ± 0.7
Steady state exercise	40.4 ± 2.0	33.6 ± 4.1	43.2 ± 1.7
Peak exercise	52.5 ±2.2	43.6 ± 2.8	52.4 ± 3.5

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p \geq 0.05$. VCO_2, carbon dioxide output; VO_{2STPD}, oxygen uptake.

Table III-10. Physiologic dead space/tidal volume ratio, respiratory exchange ratio and end-tidal carbon dioxide tension changes occurring during exercise in Control subjects.

	LSD	NSD	HSD
V_D/V_T			
Rest	0.30 ± 0.02	0.30 ± 0.02	0.30 ± 0.02
Steady state exercise	0.21 ± 0.01	0.22 ± 0.02	0.20 ± 0.01
Peak Exercise	0.15 ± 0.01	0.14 ± 0.02	0.12 ± 0.01
RER			
Rest	0.88 ± 0.06	0.83 ± 0.07	0.80 ± 0.06
Steady state exercise	0.86 ± 0.07	0.90 ± 0.06	0.86 ± 0.07
Peak exercise	0.93 ± 0.06	0.93 ± 0.07	0.92 ± 0.06
$P_{ET}CO_2$ (mmHg)			
Rest	29.5 ± 1.0	27.6 ± 1.8	32.0 ± 1.5
Steady state exercise	37.0 ± 1.4	36.1 ± 1.4	37.1 ± 1.0
Peak exercise	34.1 ±1.4	32.3 ± 1.0	35.7 ± 0.8

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p \geq 0.05$. V_D/V_T, physiologic dead space/tidal volume ratio; RER, respiratory exchange ratio (VCO_2/VO_2); $P_{ET}CO_2$, the PCO_2 of the respired gas determined at the end of an exhalation.

Table III-11. Arterial oxygen saturation changes occurring during exercise in Control subjects.

	LSD	NSD	HSD
S_aO_2 (%)			
Rest	95.9± 1.1	96.0 ± 1.1	92.0 ± 2.6
Steady state exercise	91.0 ± 3.4	91.0 ± 2.8	93.4 ± 2.7
Peak Exercise	94.0 ± 1.5	94.5 ± 1.1	92.8 ± 4.1

Values are mean ± SEM. There are no significant differences in S_aO_2 value by diet or exercise intensity, $p \geq 0.05$. S_aO_2, arterial blood oxygen saturation.

Table III-12. Cardiovascular changes occurring during exercise in EIA subjects.

	LSD	NSD	HSD
HR (bpm)			
Rest	81 ± 4.9	89 ± 6.2	85 ± 6.6
Steady state exercise	161 ± 2.2*	163 ± 2.2	164 ± 2.3
Peak Exercise	176 ± 3.6	183 ± 6.2	177 ± 6.2
HR %predicted max (bpm)			
Rest	46 ± 3.8	48 ± 3.1	45 ± 3.4
Steady state exercise	87 ± 1.5	88 ± 1.4	88 ± 1.1
Peak exercise	94 ± 2.7	99 ± 2.8	99 ± 2.7
O_2 Pulse (ml/beat)			
Rest	3.0 ± 0.5	2.6 ± 0.6	2.2 ± 0.4*
Steady state exercise	13.0 ± 1.3*	13.0 ± 1.2*	11.8 ± 1.0*
Peak Exercise	14.8 ± 1.5*	16.1 ± 1.4*	14.1 ± 1.3*

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. There were no significant differences in ventilatory and metabolic values by diet, $p>0.05$. HR, heart rate; HR%, HR as a percentage of maximum heart rate (220-Age); O_2 Pulse, oxygen pulse (VO_2/HR).

Table III-13. Breathing pattern and exercise ventilation changes occurring during exercise in EIA subjects.

	LSD	NSD	HSD
V_T (L)			
Rest	0.67 ± 0.04[a]	0.67 ± 0.04[a]	0.61 ± 0.05[a]
Steady state exercise	1.4 ± 0.12*[a]	1.6 ± 0.18*[a]	1.9 ± 0.19*[b]
Peak Exercise	1.6 ± 0.24*[a]	2.0 ± 0.28*[b]	2.3 ± 0.23*[c]
f_b (breaths/min)			
Rest	15.6 ± 1.2[a]	15.6 ± 1.3[a]	17.9 ± 1.9[a]
Steady state exercise	44.8 ± 1.7*[a]	40.4 ± 3.1*[a]	33.6 ± 2.1[b]
Peak exercise	58.4 ±2.9*[a]	51.5 ± 2.4*[b]	43.4 ± 2.0*[c]
$V_{E\,BTPS}$ (L/min)			
Rest	10.5 ± 0.3[a]	10.5 ± 0.5[a]	10.9 ± 0.5[a]
Steady state exercise	64.1 ± 4.2[a]	64.7 ± 5.2[a]	64.0 ± 5.7[a]
Peak exercise	93.4 ± 11.2[a]	103 ± 11.2[a]	97.5 ± 10.4[a]

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. Letters [a,b,c] refer to comparisons by diet within specific variable; different letters designate significant difference, $p<0.05$. V_T, tidal volume; f_b, respiratory frequency; $V_{E\,BTPS}$, minute ventilation.

Table III-14. Oxygen uptake and carbon dioxide output changes occurring during exercise in EIA subjects.

	LSD	NSD	HSD
VCO_{2STPD} (L/min)			
Rest	0.19 ± 0.02	0.18 ± 0.01	0.16 ± 0.03
Steady state exercise	1.78 ± 0.19	1.8 ± 0.23	1.67 ± 0.23
Peak Exercise	2.4 ± 0.38*	2.6 ± 0.45	2.3 ± 0.34*
VO_{2STPD} (L/min)			
Rest	0.29 ± 0.02	0.23 ± 0.02	0.19 ± 0.02
Steady state exercise	2.10 ± 0.23	2.12 ± 0.30[a]	1.94 ± 0.27*
Peak Exercise	2.61 ± 0.36*	2.94 ± 0.47	2.50 ± 0.37*
VO_{2STPD} (ml/min/kg)			
Rest	3.6 ± 0.2	4.0 ± 0.3	3.2 ± 0.3*
Steady state exercise	36.2 ± 3.2	35.8 ± 2.8	32.5 ± 2.6*
Peak exercise	45.3 ±5.2*	44.1 ± 5.7	41.6 ± 3.8*

Values are ± mean SEM. *$p<0.05$ compared to respective value in Control subjects. There were no significant differences in ventilatory and metabolic values by diet, $p>0.05$. VCO_2, carbon dioxide output; VO_{2STPD}, oxygen uptake.

Table III-15. Physiologic dead space/tidal volume ratio, respiratory exchange ratio and end-tidal carbon dioxide tension changes occurring during exercise in EIA subjects.

	LSD	NSD	HSD
V_D/V_T			
Rest	0.33 ± 0.02[a]	0.32 ± 0.02[a]	0.36 ± 0.03[a]
Steady state exercise	0.19 ± 0.01[a]	0.22 ± 0.02[a]	0.27 ± 0.02*[b]
Peak Exercise	0.13 ± 0.02[a]	0.18 ± 0.02[b]	0.24 ± 0.01*[c]
RER			
Rest	0.79 ± 0.07*[a]	0.78 ± 0.08*[a]	0.84 ± 0.06*[b]
Steady state exercise	0.84 ± 0.08*[a]	0.85 ± 0.07*[a]	0.86 ± 0.05*[a]
Peak exercise	0.92 ± 0.06[a]	0.88 ± 0.06*[a]	0.92 ± 0.07[a]
$P_{ET}CO_2$ (mmHg)			
Rest	26.1 ± 1.3[a]	28.1 ± 0.8[a]	25.3 ± 1.8*[a]
Steady state exercise	32.6 ± 1.1[a]	33.0 ± 1.1[a]	32.3 ± 1.1*[a]
Peak exercise	31.3 ±1.3[a]	30.1 ± 1.3[a]	31.1 ± 1.5*[a]

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. Letters [a,b,c] refer to comparisons by diet within specific variable; different letters designate significance difference, $p<0.05$. V_D/V_T, physiologic dead space/tidal volume ratio; RER, respiratory exchange ratio (VCO_2/VO_2); $P_{ET}CO_2$, the PCO_2 of the respired gas determined at the end of an exhalation.

Table III-16. Arterial oxygen saturation changes occurring during exercise in EIA subjects.

	LSD	NSD	HSD
S_aO_2 (%)			
Rest	96.8 ± 1.5	97.2 ± 1.4	96.0 ± 1.4
Steady state exercise	95.4 ± 2.9	94.9 ± 2.1	94.6 ± 1.4
Peak Exercise	95.7 ± 1.2	94.7 ± 1.5	95.7 ± 1.6

Values are mean ± SEM. There are no significant differences in S_aO_2 value by diet and exercise intensity, or compared to respective value in Control subjects, $p>0.05$. S_aO_2, arterial blood oxygen saturation.

Analysis of Variance for 2 X 2 Crossover Design: FEV1 @ 10 min post-exercise. EIA subjects.					
Source	*d. f.*	*Sum of Squares*	*Mean Square*	*F value*	*P value*
Between-subjects:					
Carry-over	1	0.526	0.5260	0.0582	0.813
B.S. residual	6	9.038	1.5063		
Within-subjects:					
Direct Treatments (adjusted for Periods)	1	0.922	0.9216	91.89	0.000
Periods (adjusted for Treatments)	1	0.021	0.0210	0.969231	0.343
W-S residual	6	0.130	0.0217		
		10.637			
Total	15	10.636			

Table III-17. Analysis of variance for a 2 x 2 crossover design to assess carry-over effects for FEV_1 at 10 minutes post-exercise in EIA subjects.

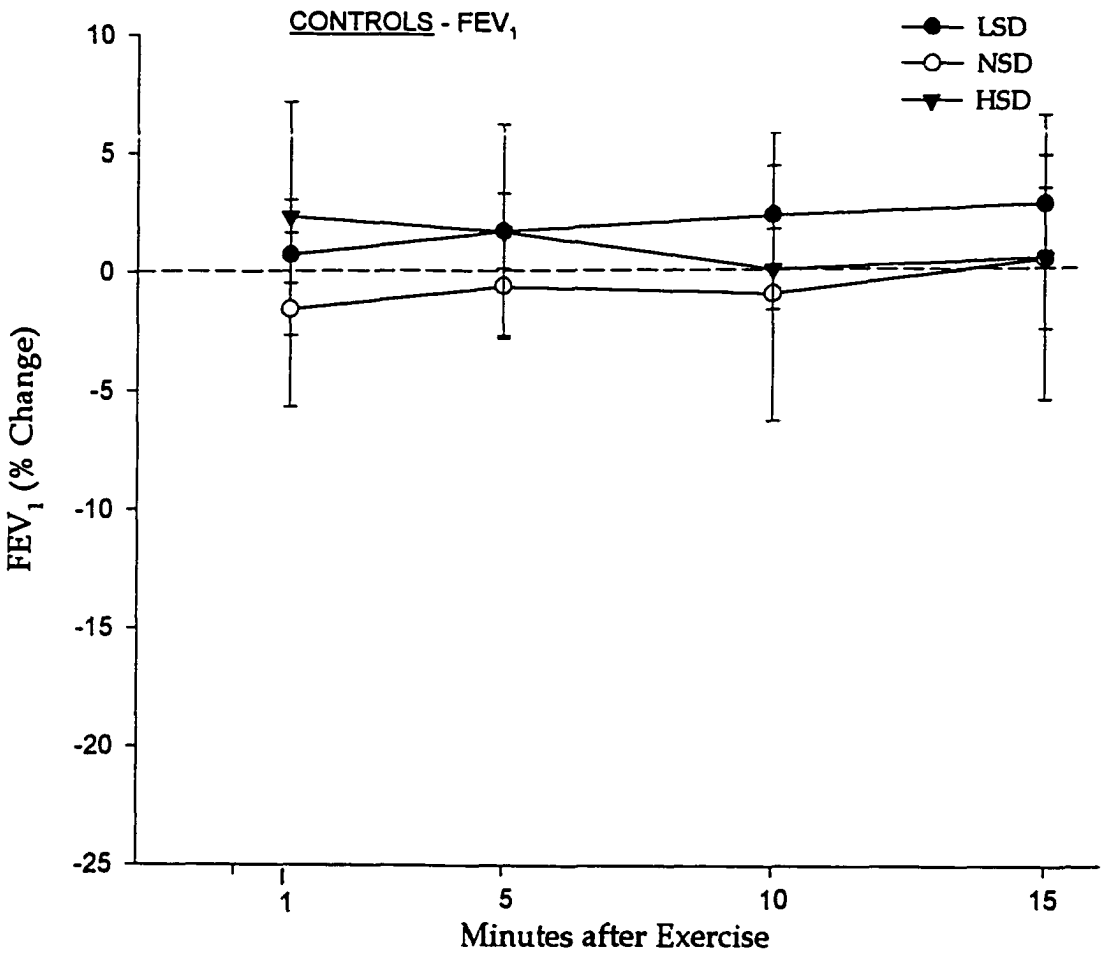

Figure III-1. Mean percentage change in FEV_1 pre- to post-exercise in Control subjects.

Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$).

LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet

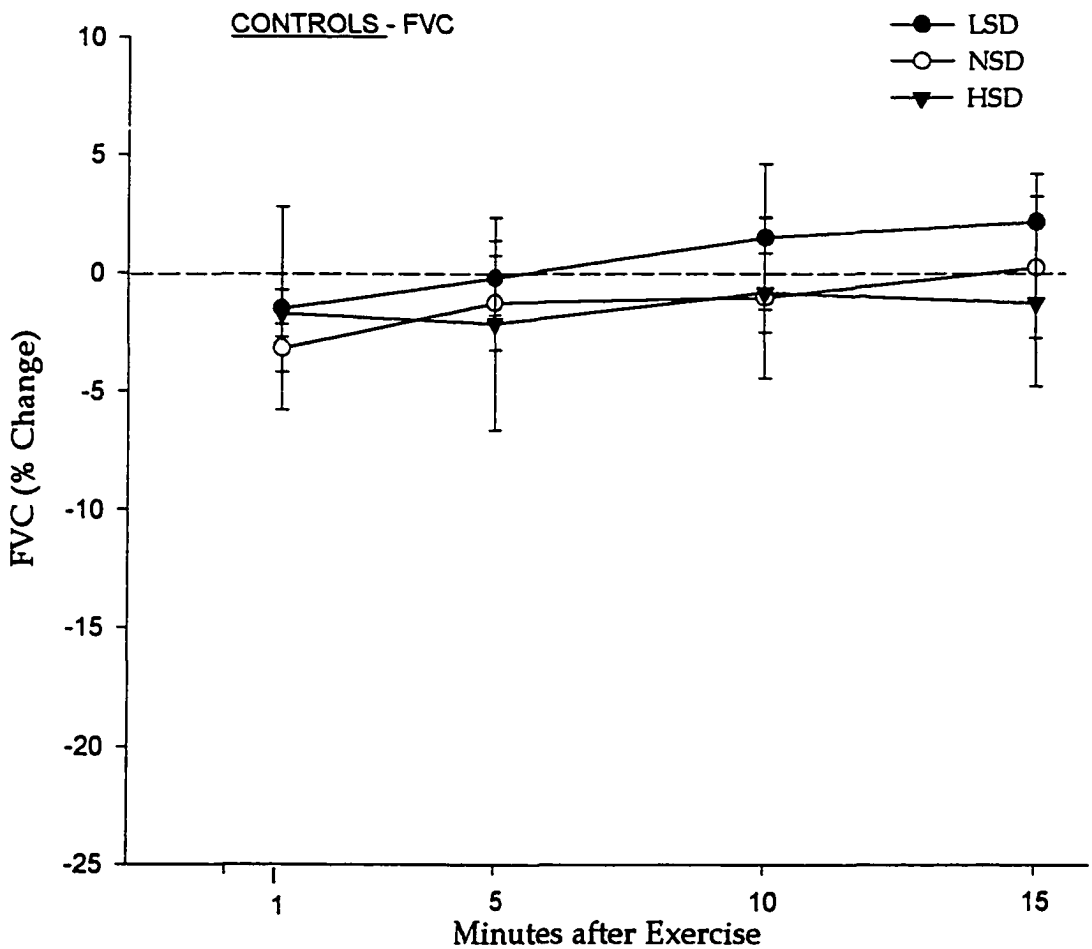

Figure III-2. Mean percentage change in FVC pre- to post-exercise in Control subjects.

Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$).

LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

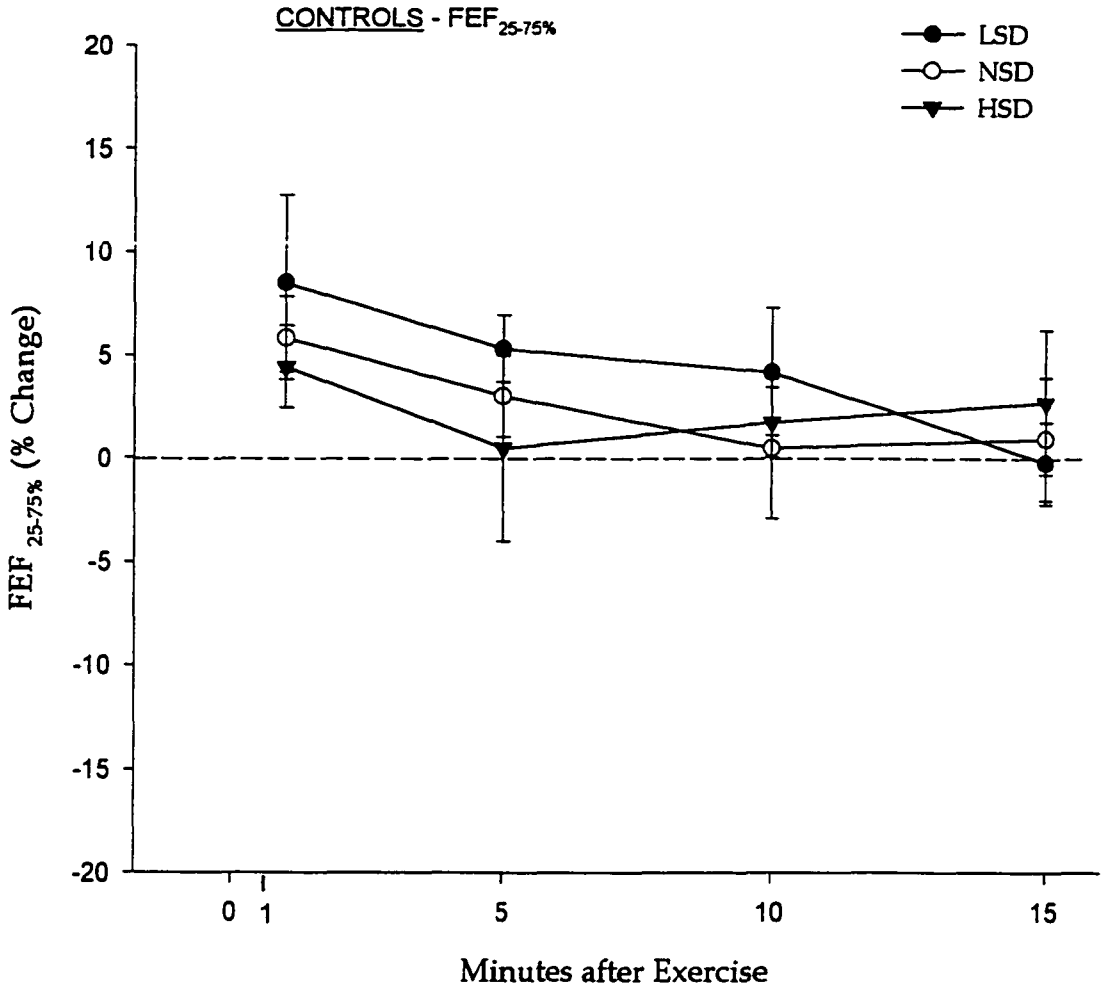

Figure III-3. Mean percentage change in FEF $_{25-75\%}$ pre- to post-exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

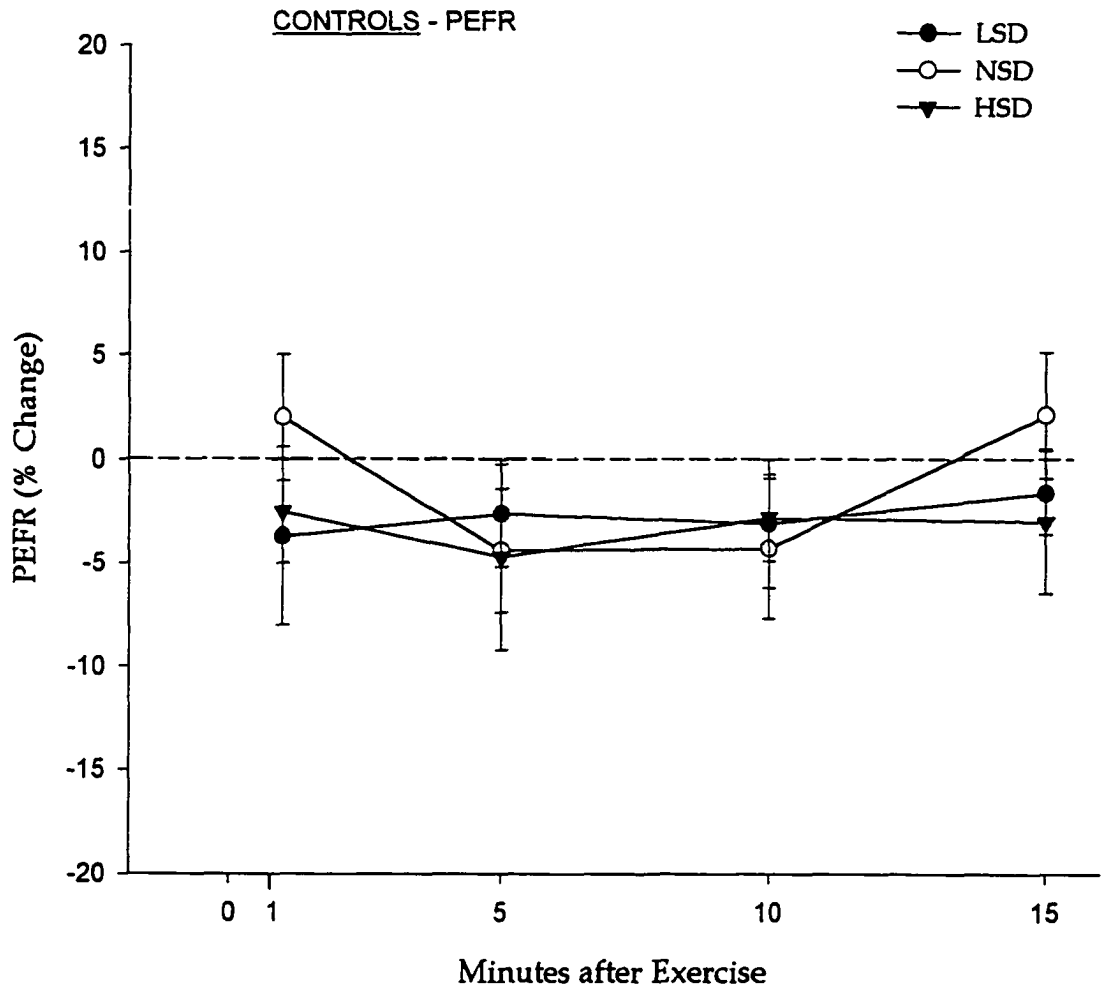

Figure III-4. Mean percentage change in PEFR pre- to post-exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

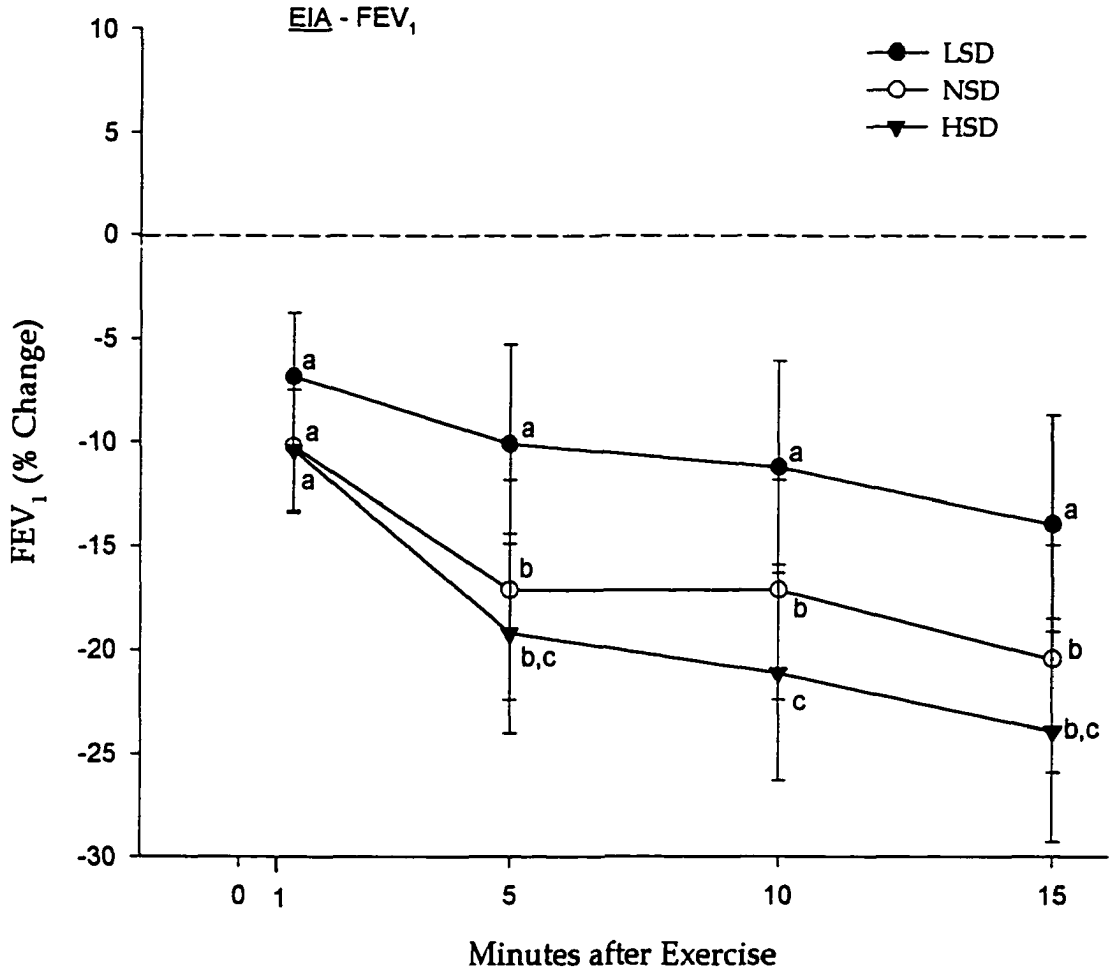

Figure III-5. Mean percentage change in FEV_1 pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

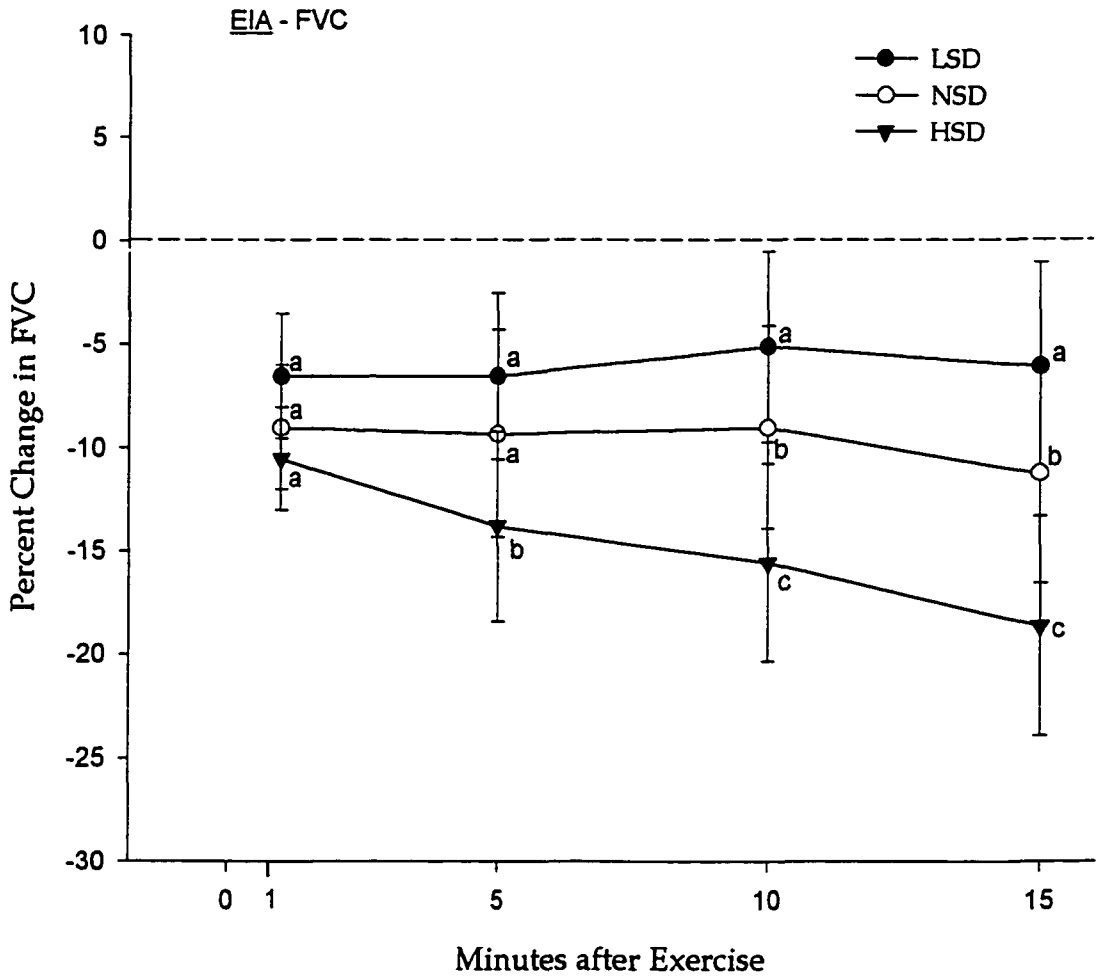

Figure III-6. Mean percentage change in FVC pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$) differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

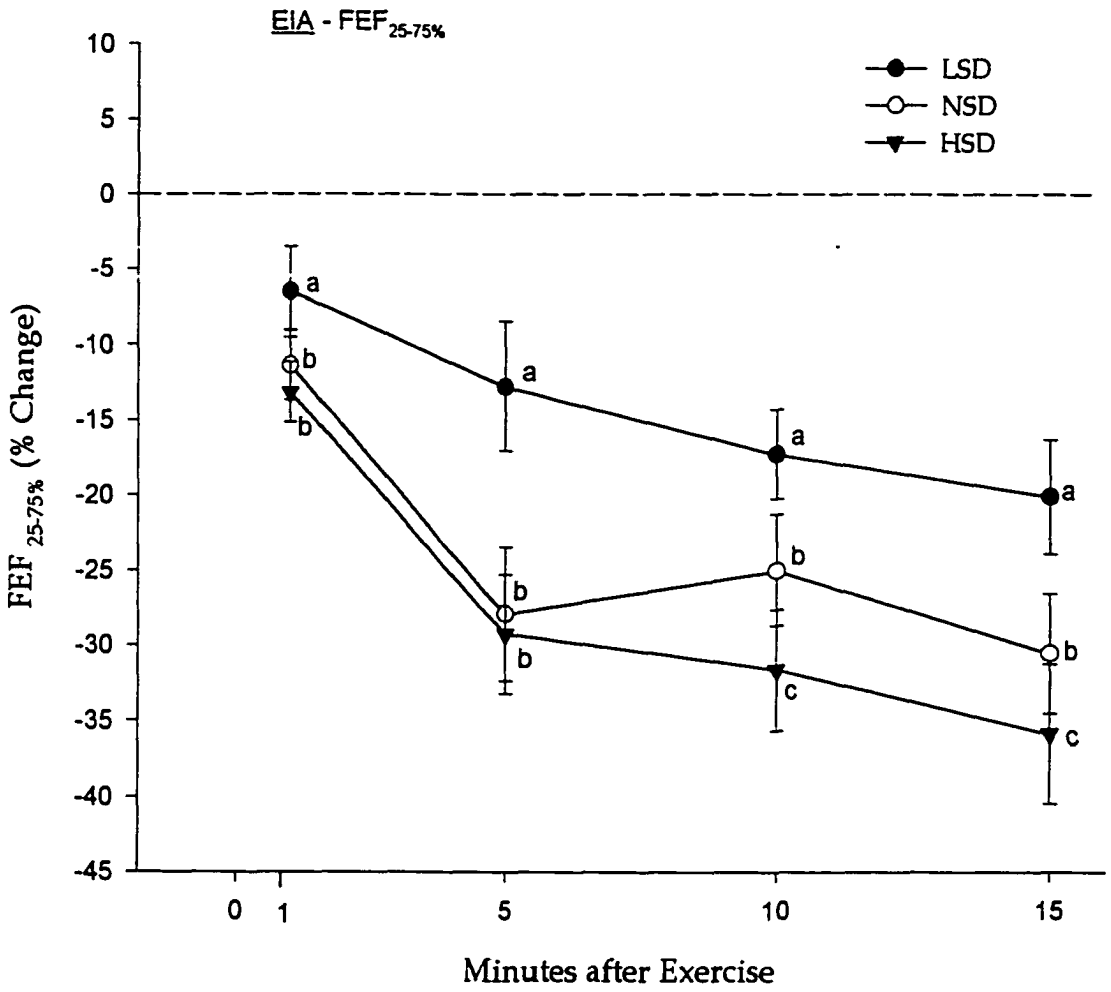

Figure III-7. Mean percentage change in FEF $_{25-75\%}$ pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

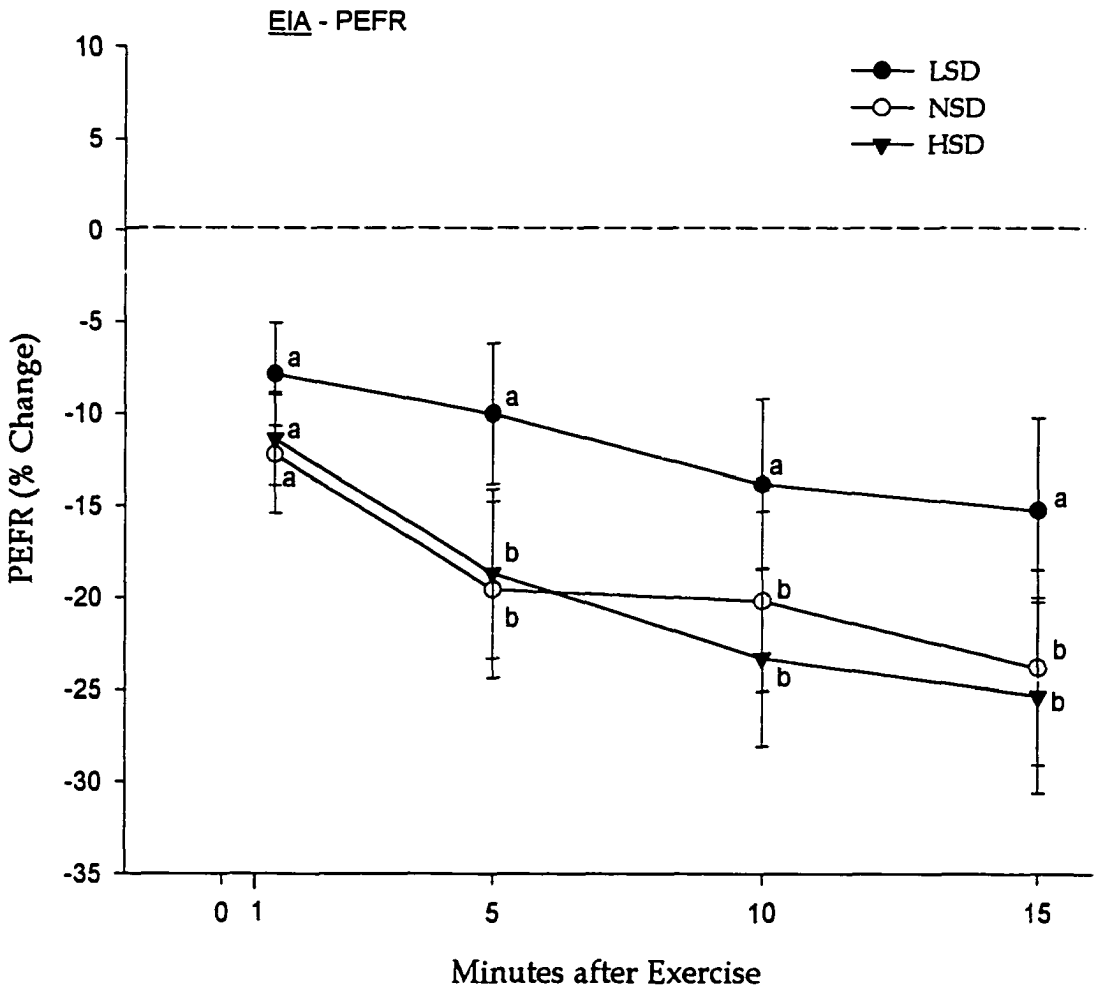

Figure III-8. Mean percentage change in PEFR pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p>0.05$). LSD-low salt diet; NSD-normal salt diet, HSD-high salt diet.

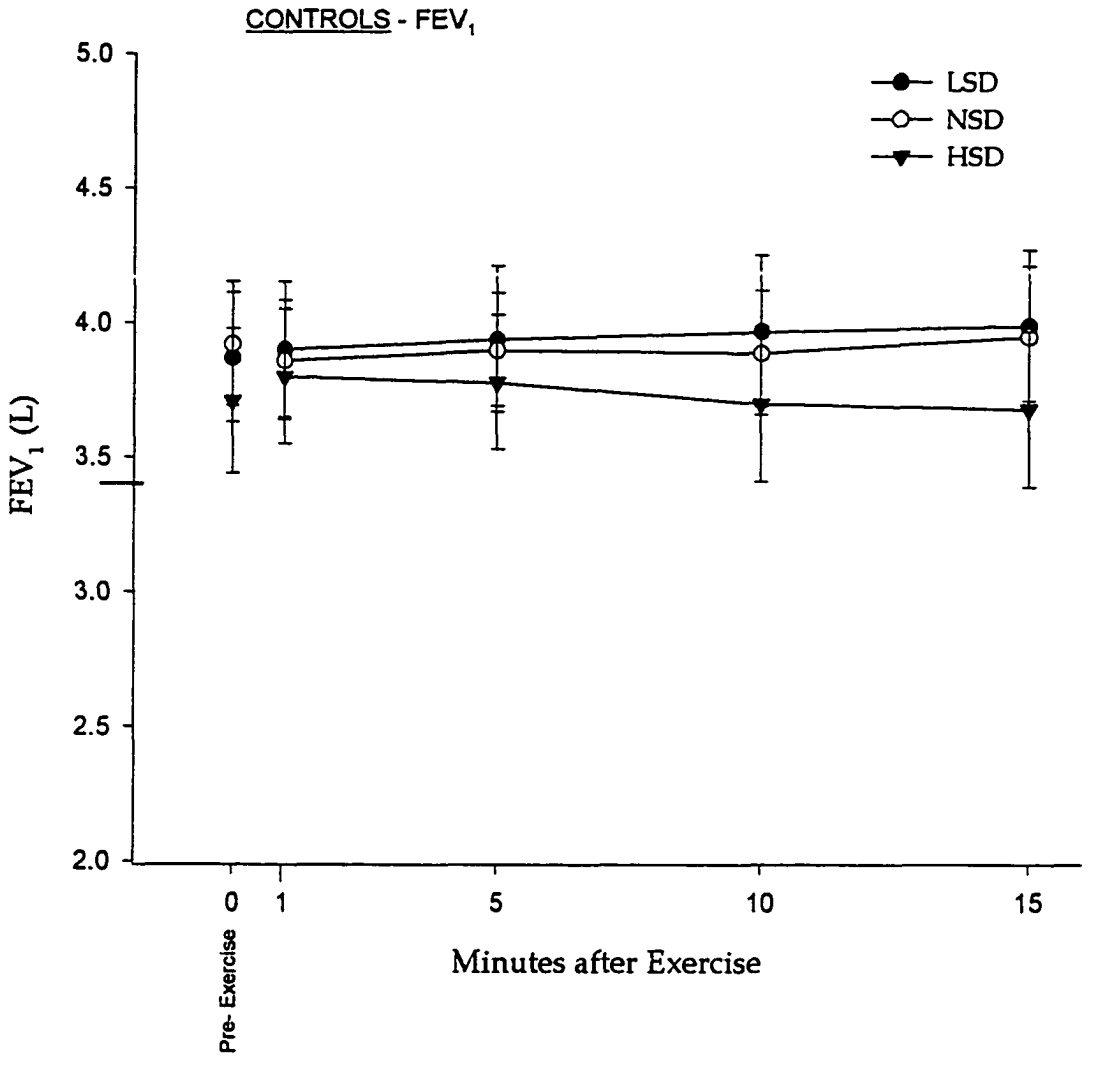

Figure III-9. Changes in FEV_1 at pre-exercise and following exercise in Control subjects.

Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$).

LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

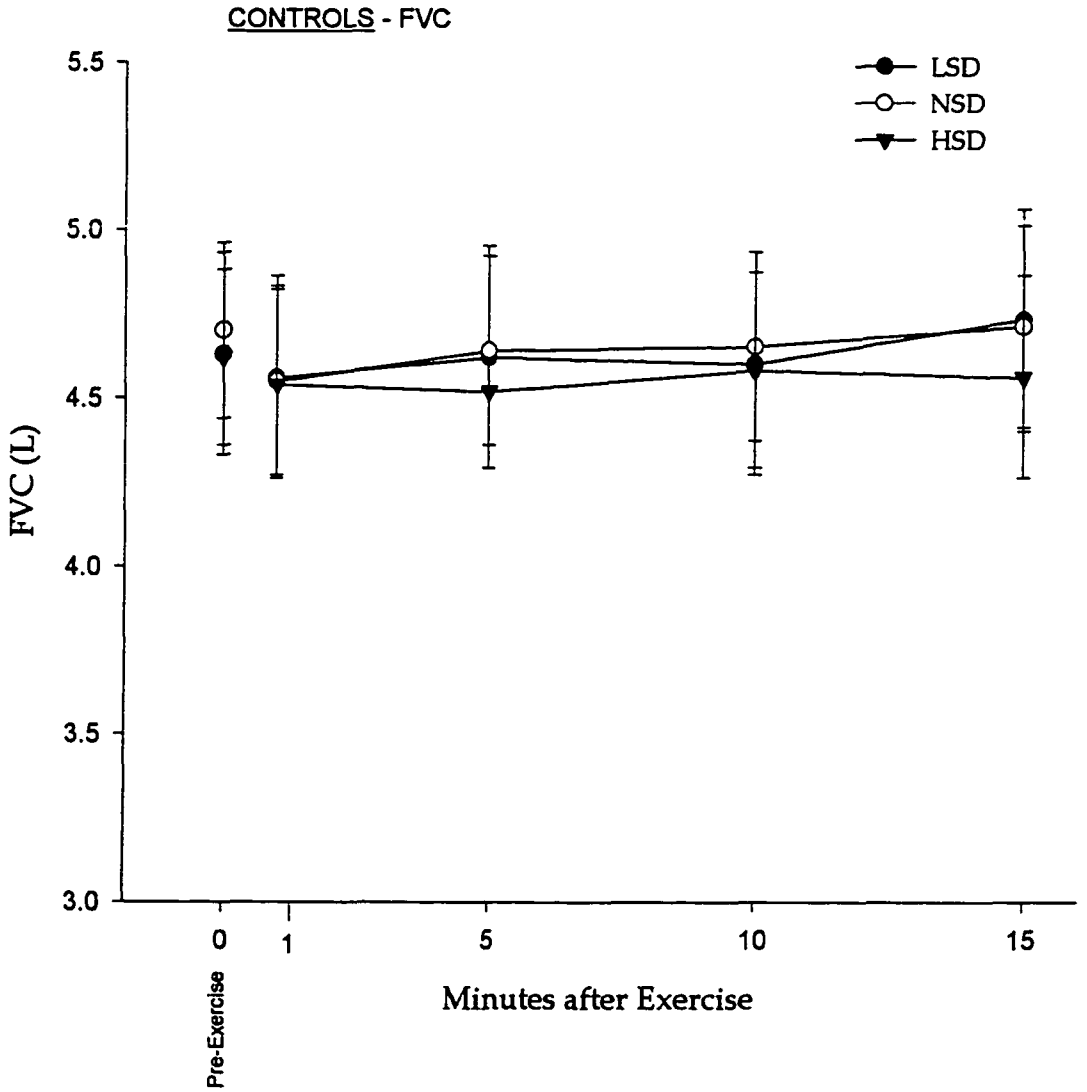

Figure III-10. Changes in FVC at pre-exercise and following exercise in Control subjects.

Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$).

LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

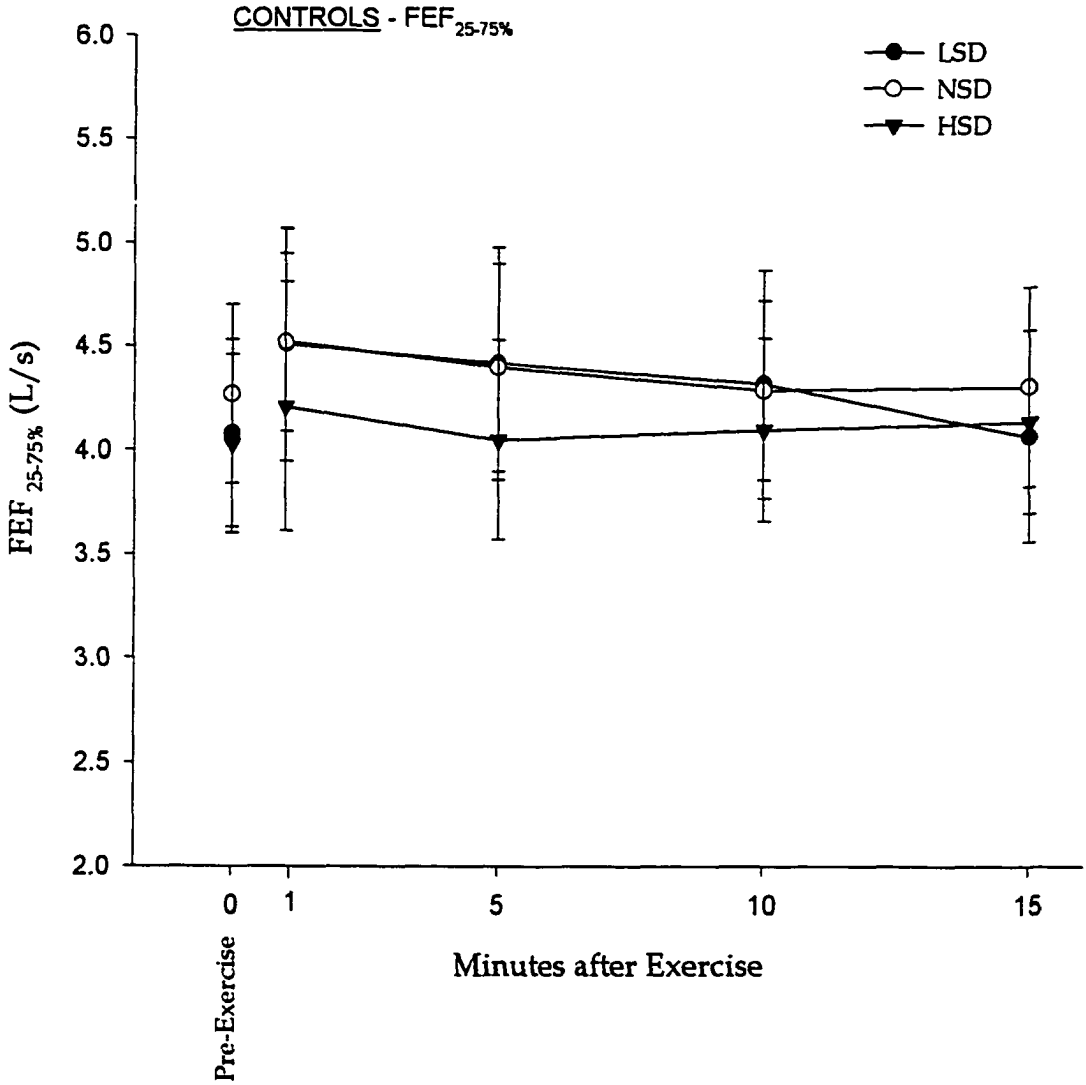

Figure III-11. Changes in $FEF_{25-75\%}$ at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

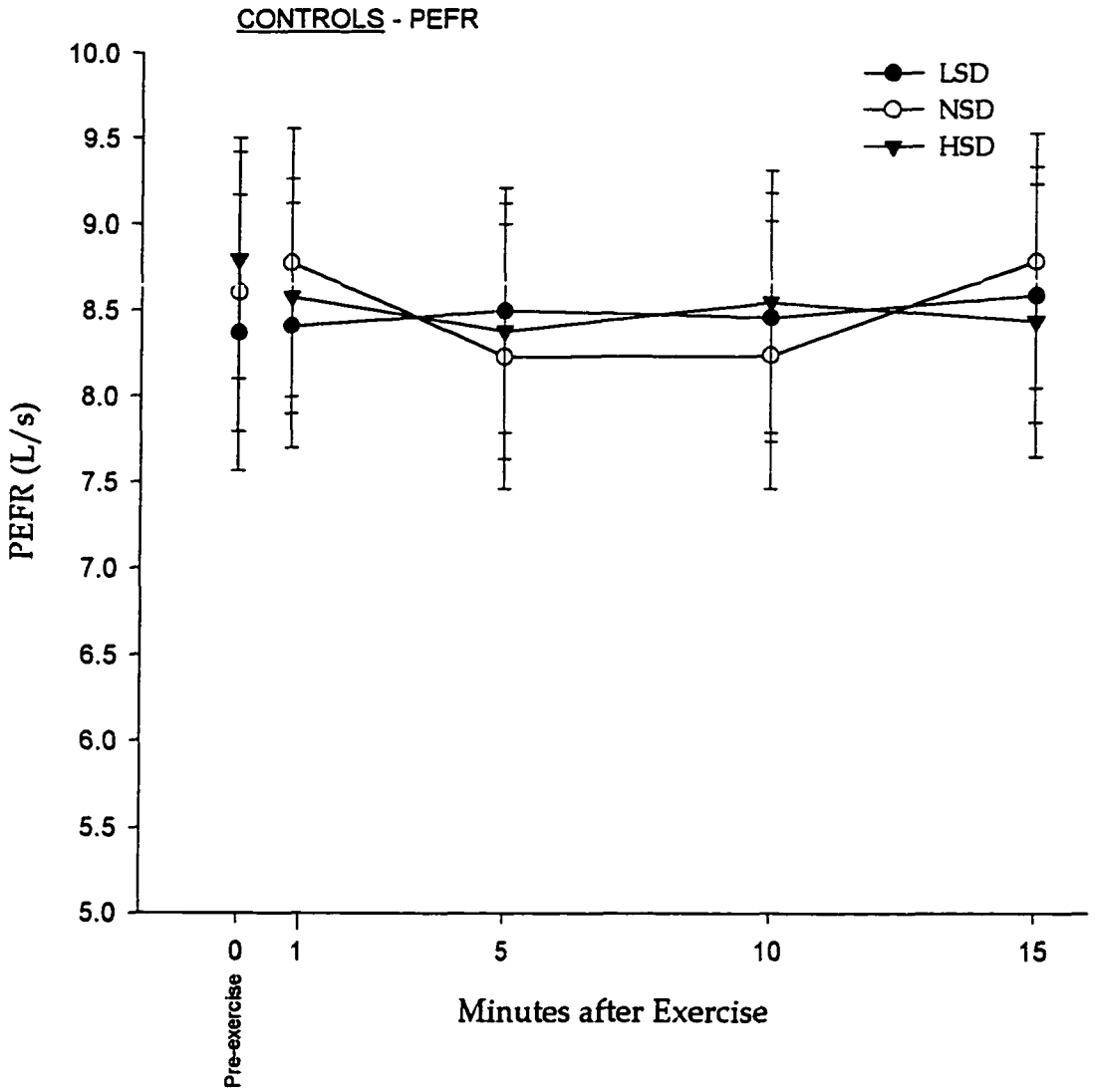

Figure III-12. Changes in PEFR at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

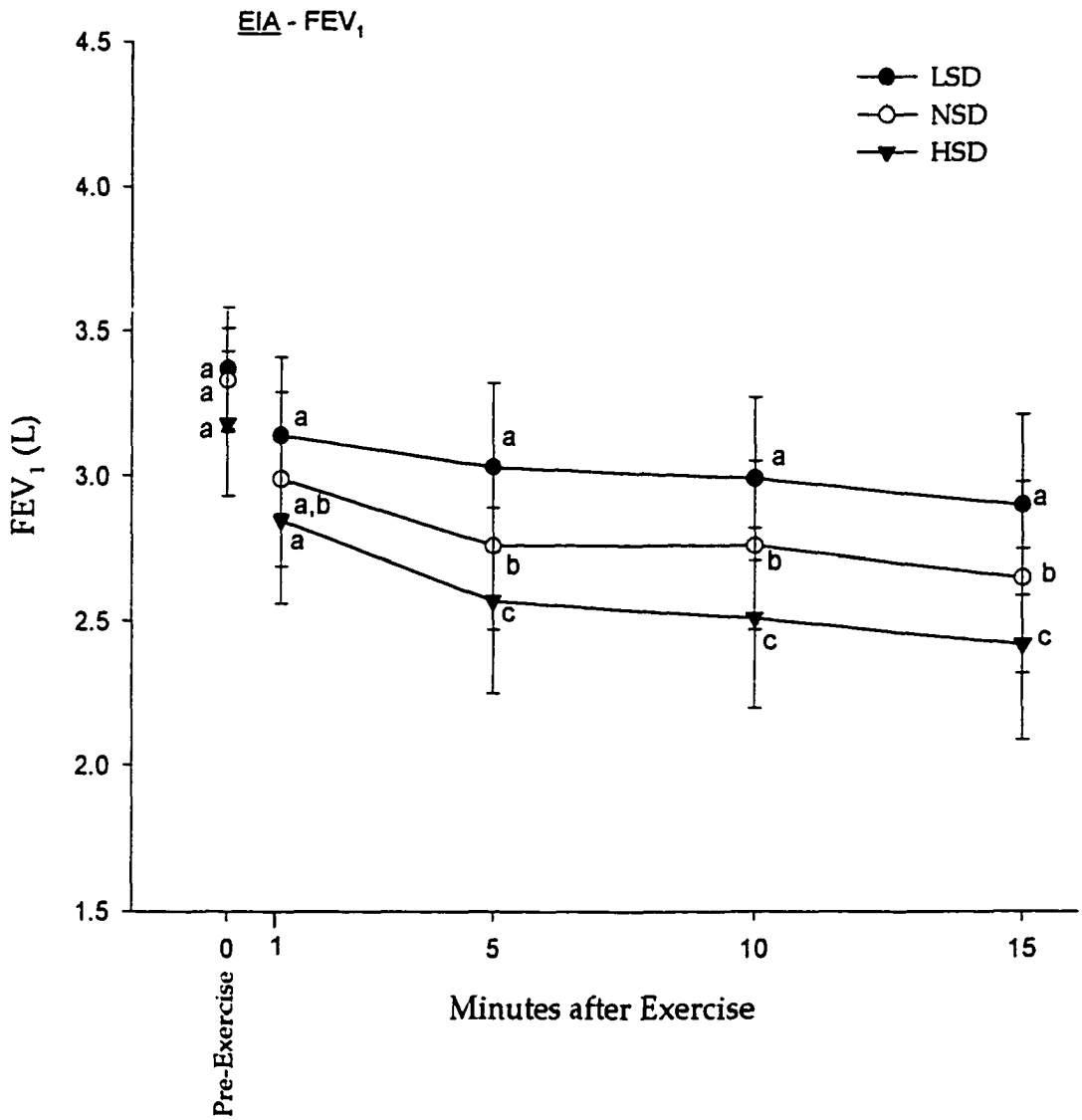

Figure III-13. Changes in FEV_1 at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet, HSD-high salt diet.

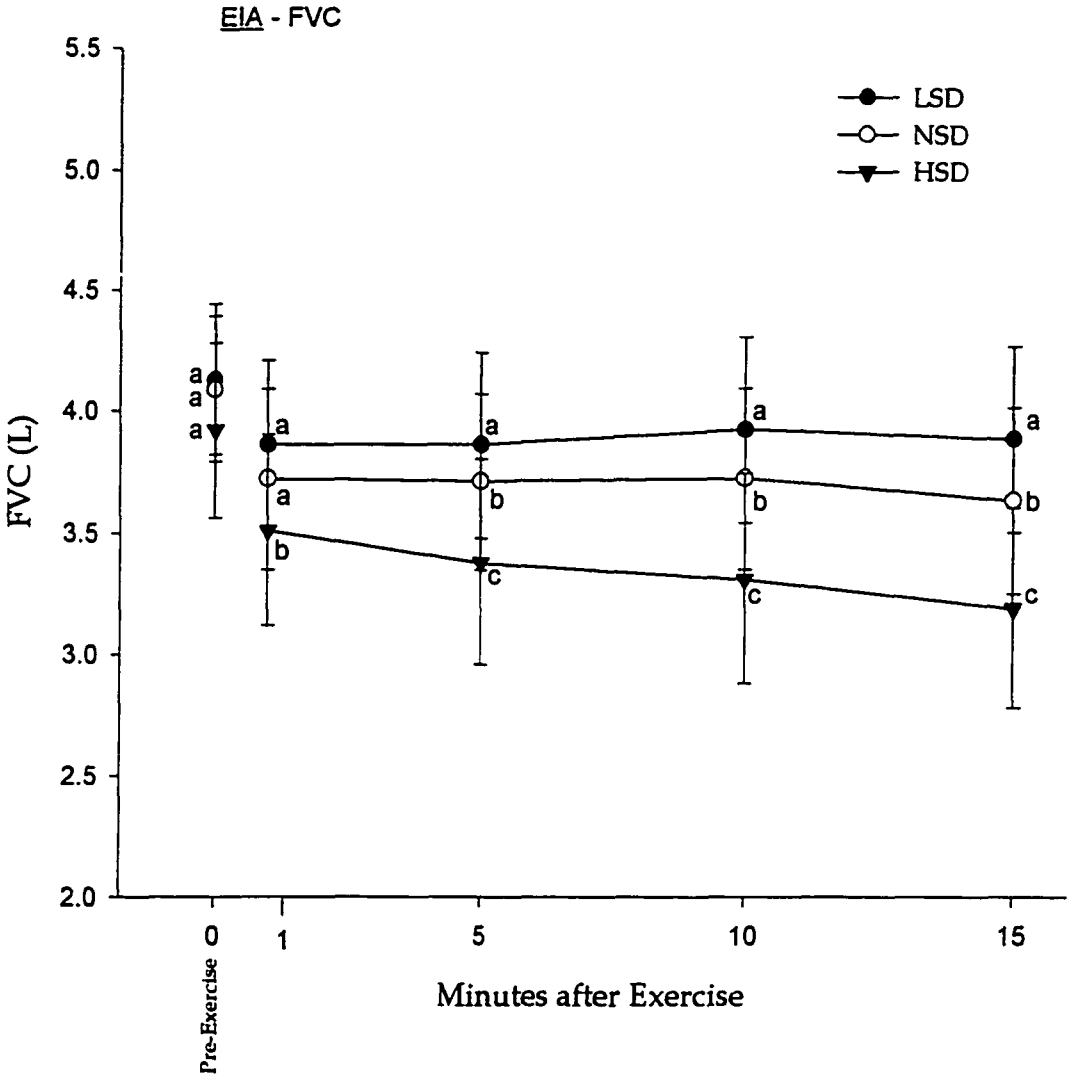

Figure III-14. Changes in FVC at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

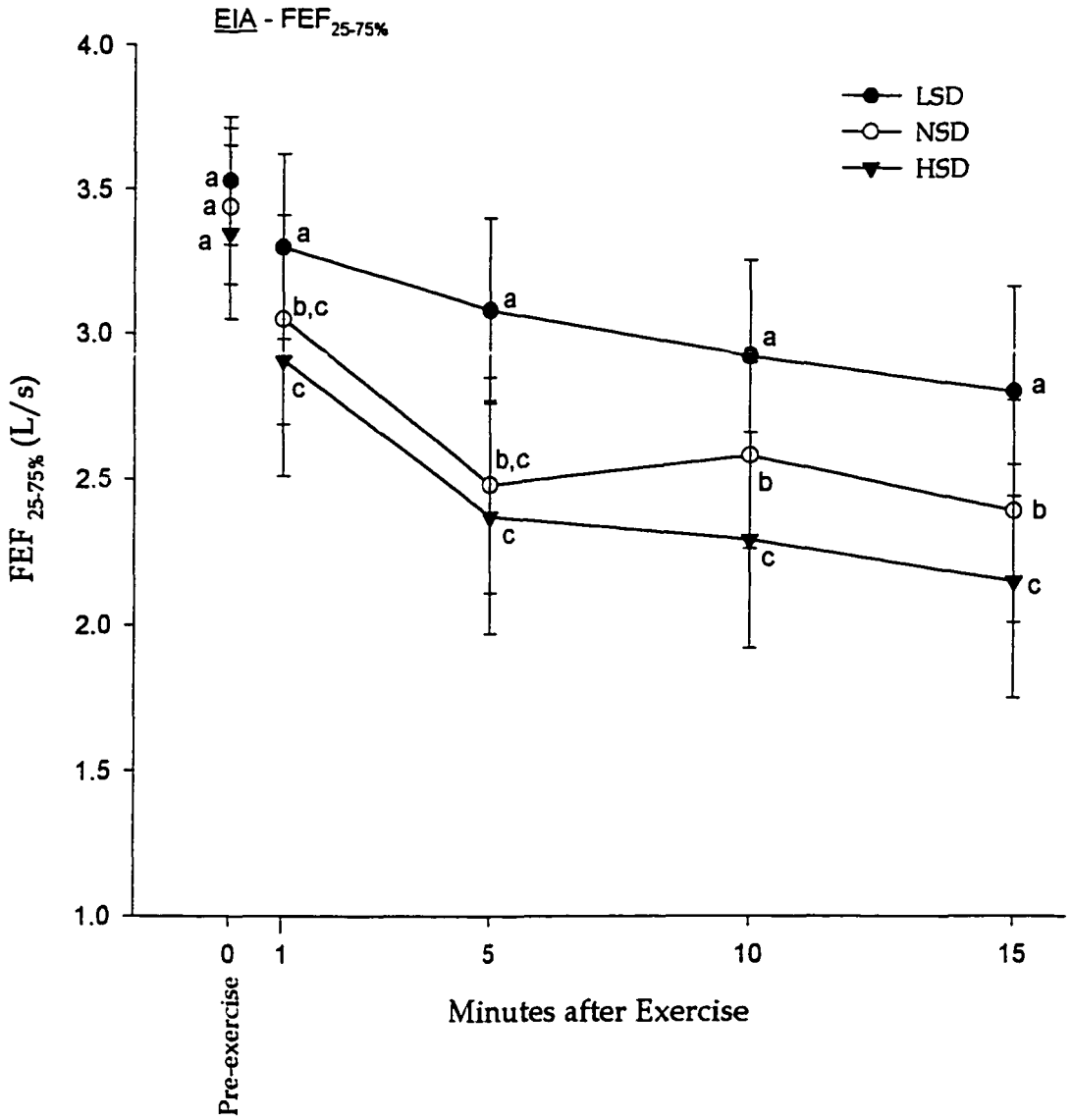

Figure III-15. Changes in $FEF_{25-75\%}$ at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salt diet.

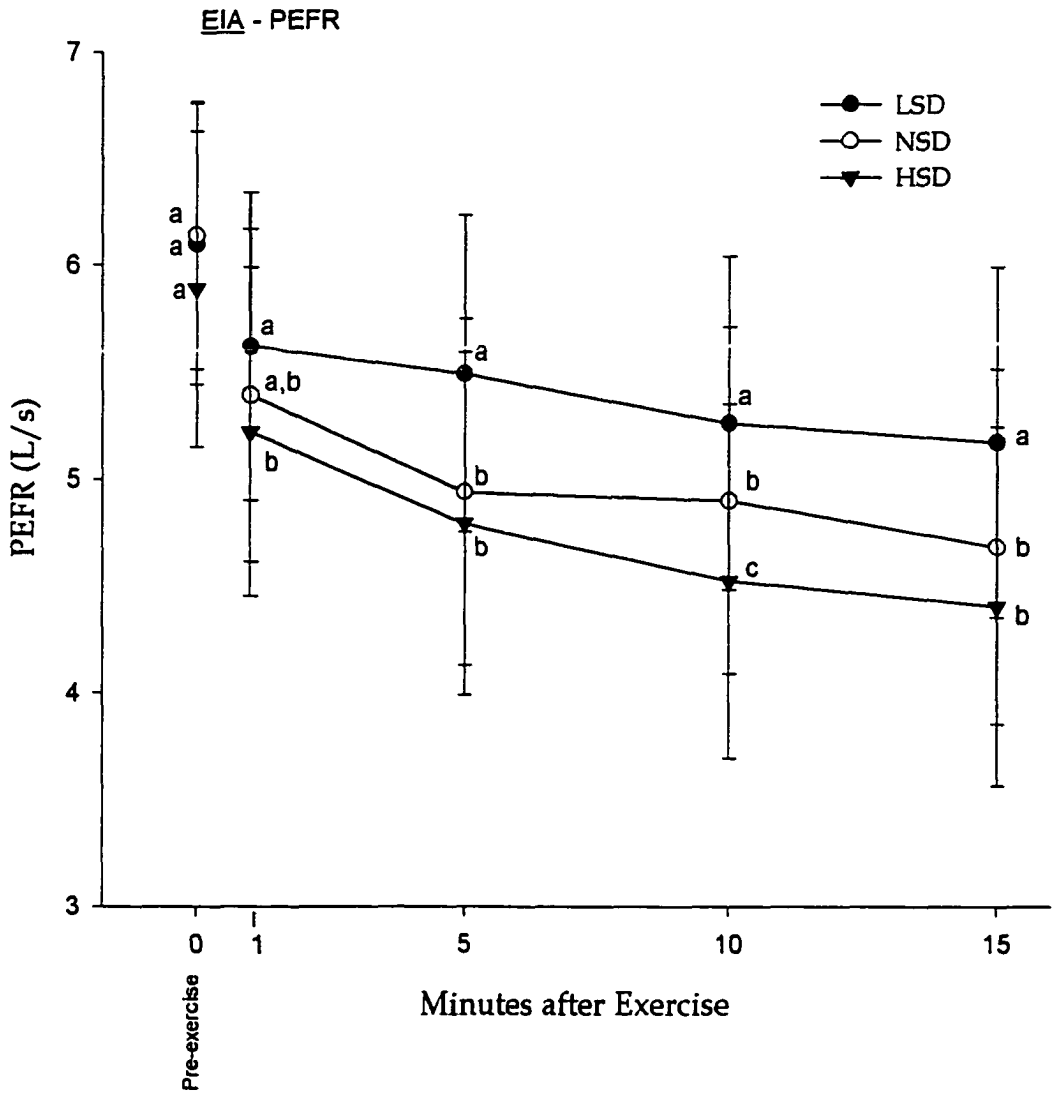

Figure III-16. Changes in PEFR at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; HSD-high salyt diet.

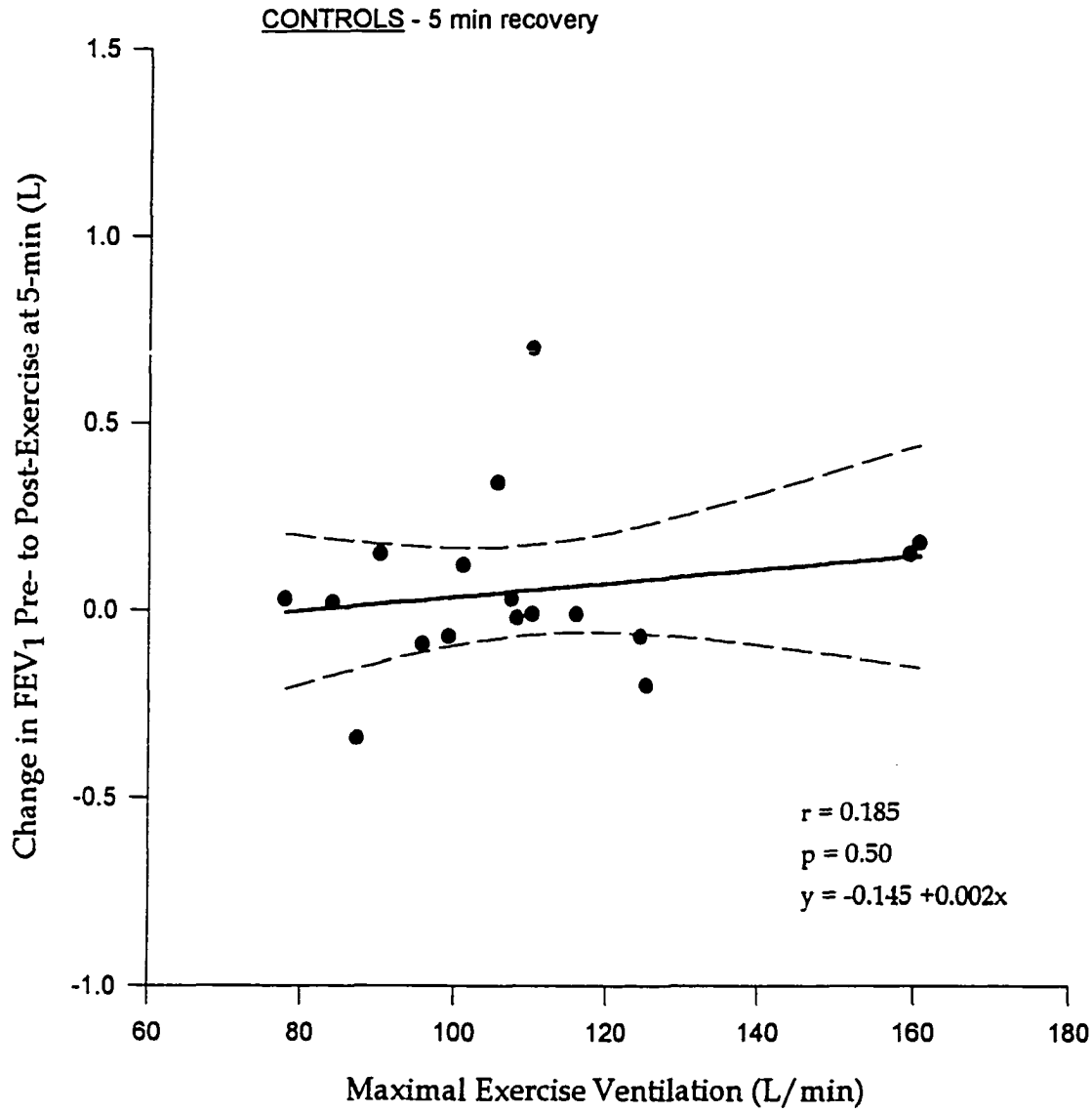

Figure III-17. Regression of maximal exercise ventilation and pre- to post exercise changes in FEV_1 at 5-minutes of recovery in Control subjects.

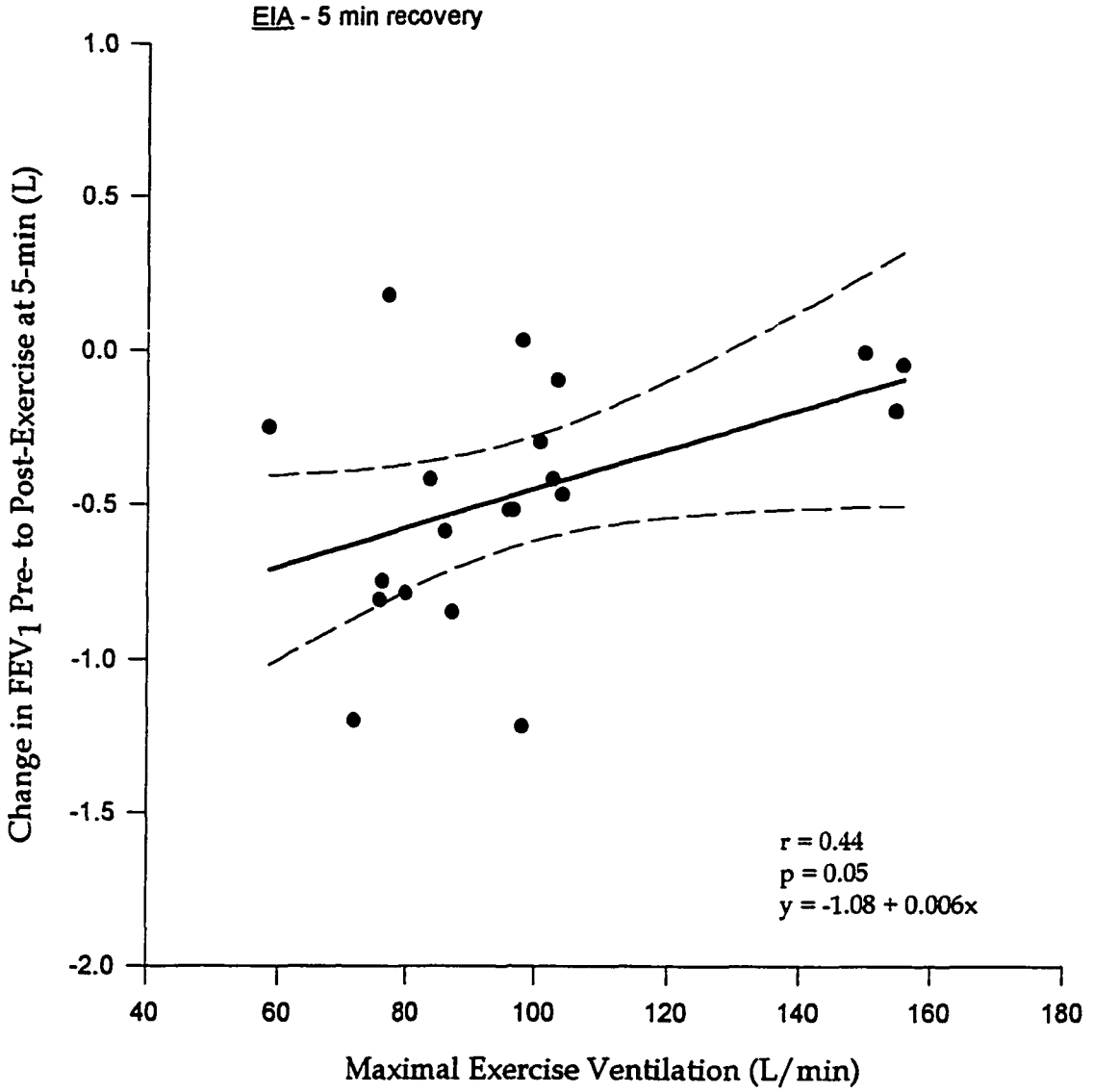

Figure III-18. Regression of maximal exercise ventilation and pre- to post-exercise change in FEV_1 at 5-minutes of recovery in EIA subjects.

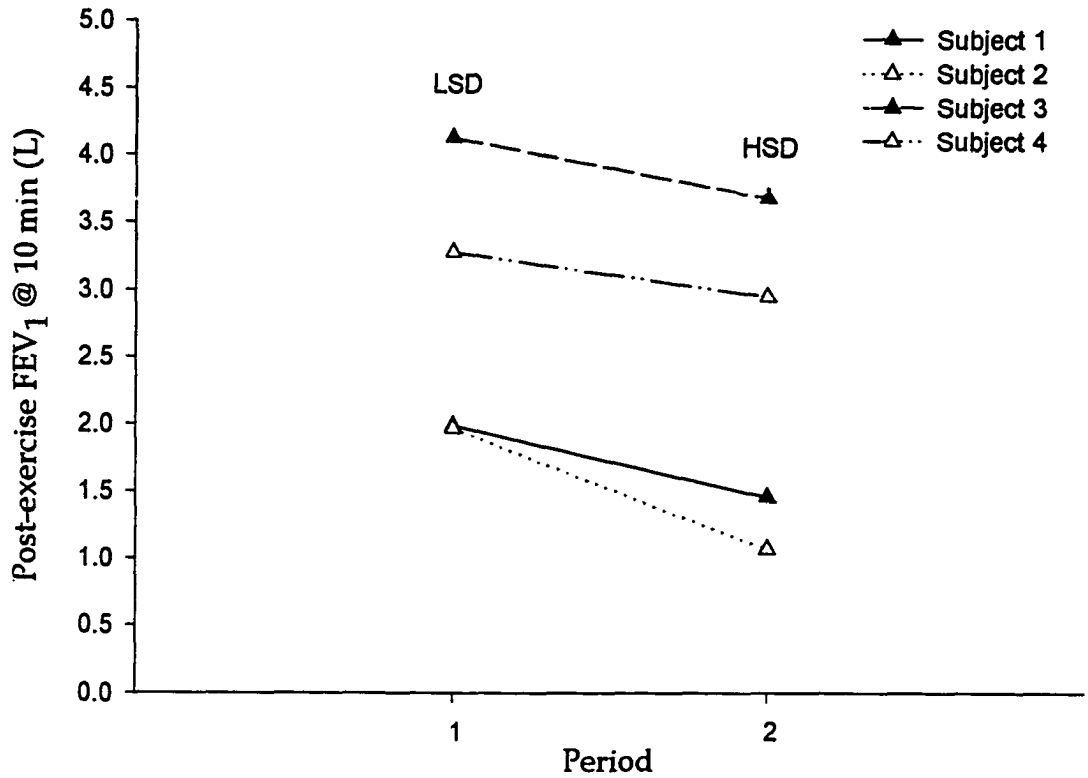

Figure III-19. EIA Group One subject profiles for FEV_1 at 10 minutes post-exercise.

Subjects started the LSD in period 1 and then crossed over to the HSD in period 2.

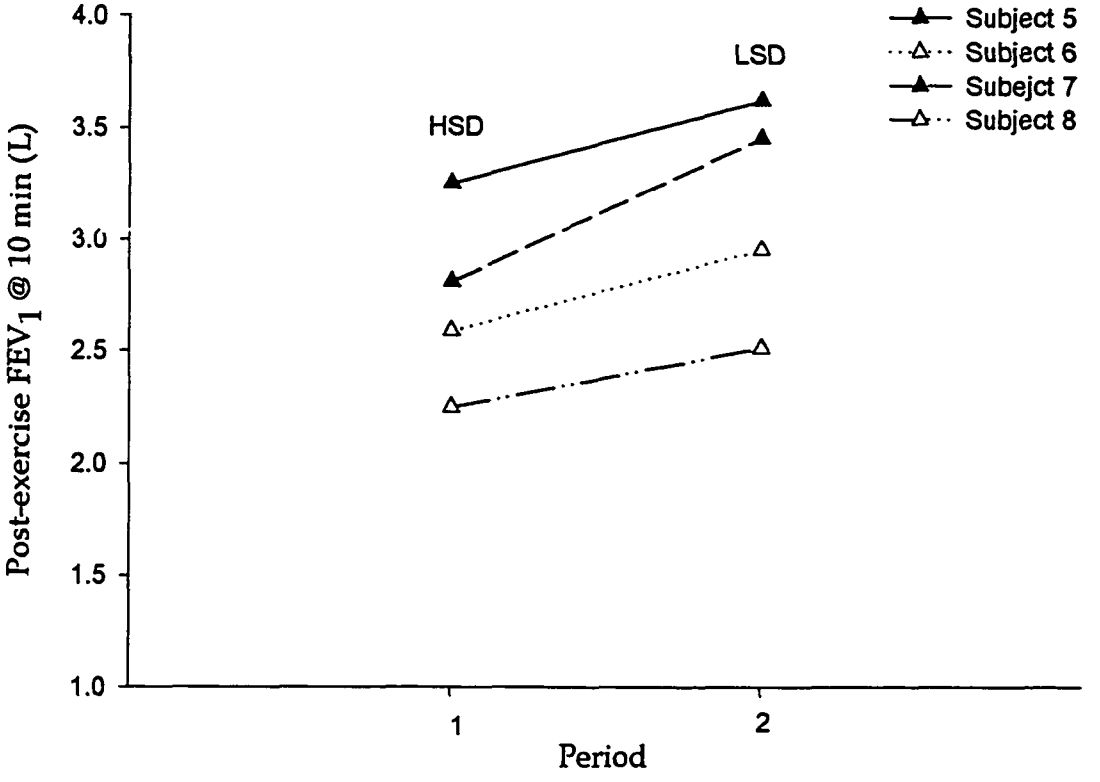

Figure III-20. EIA Group Two subject profiles for FEV_1 at 10 minutes post-exercise.

Subjects started the HSD in period 1 and then crossed over to the LSD in period 2.

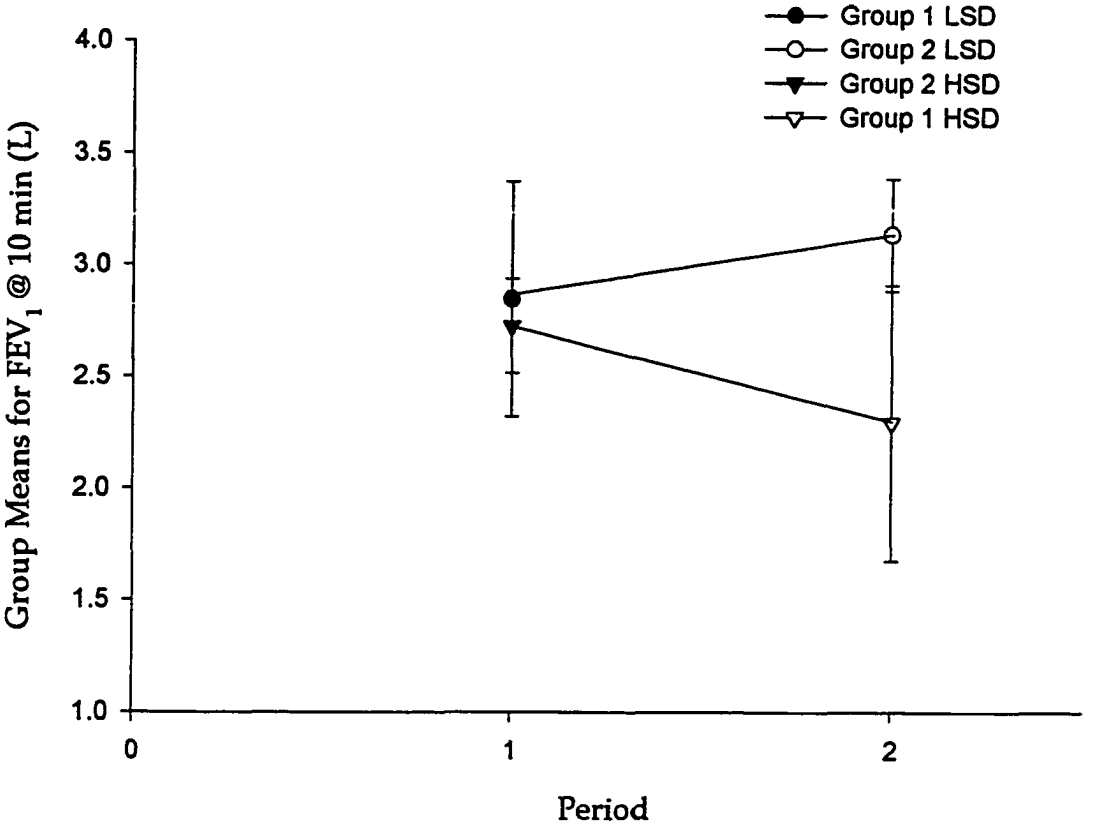

Figure III-21. Group-by-period plot for EIA subjects at 10-minutes post-exercise FEV_1.

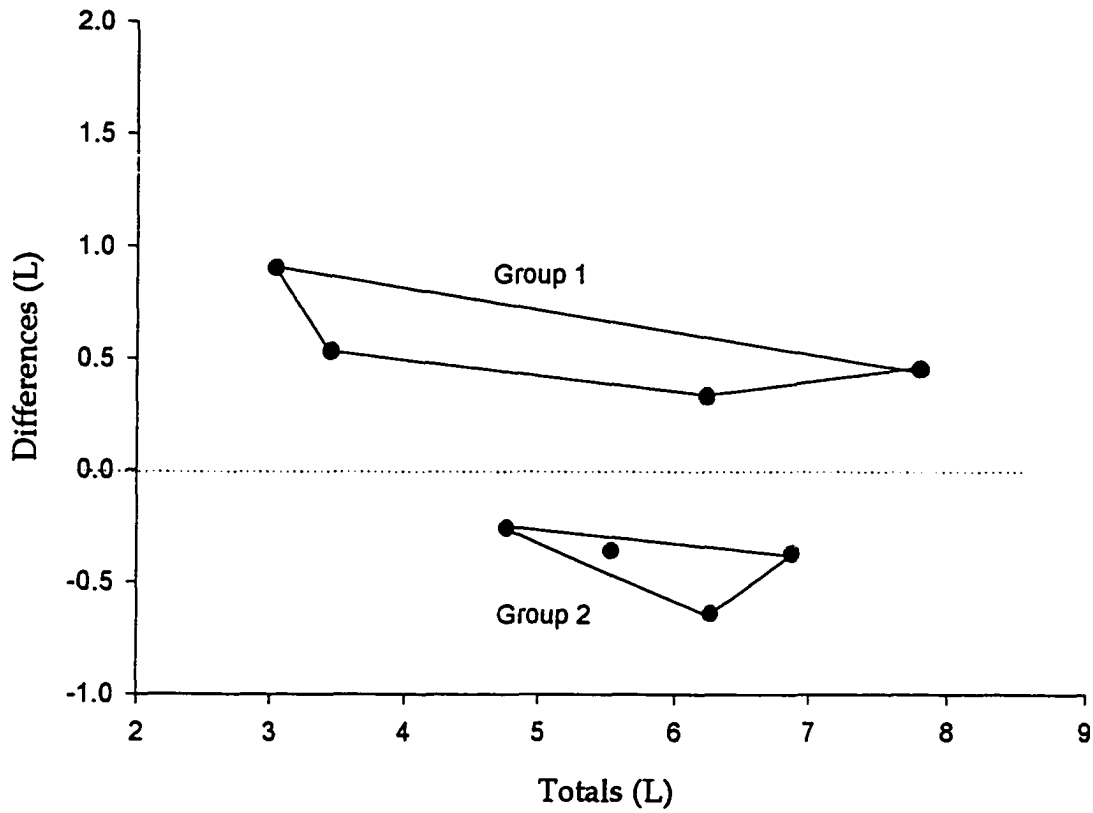

Figure III-22. Differences (period 1 minus period 2) against totals (period 1 plus period 2) plot, in order to assess carry-over effects in EIA subjects at FEV_1 at 10 minutes post-exercise.

CHAPTER IV

Dietary chloride as a possible determinant of the severity of exercise-induced asthma

Abstract

Purpose: It was observed previously that a high salt (NaCl) diet worsened and a low salt diet (LSD) improved pulmonary function in exercise-induced asthma (EIA). The purpose of this study was to determine whether it is the sodium or chloride ion in NaCl that is responsible for the observed changes in pulmonary function. This was accomplished by determining the influence of different sodium salts on post-exercise bronchoconstriction in EIA subjects. **Methods:** Eight subjects with clinically diagnosed EIA and eight subjects without EIA (Control) underwent a double-blind crossover study. Subjects entered the study on a normal salt diet (NSD), and then were placed either on a LSD (low sodium, low chloride) or a $NaHCO_3$ diet (high sodium, low chloride). A one-week washout period occurred between diets before subjects crossed over to the alternative diet. Twenty-four urine collections confirmed compliance to all diets. Pre- and -post exercise pulmonary function tests were performed at the end of each treatment period. Subjects underwent treadmill testing to volitional fatigue (peak exercise). During exercise ventilatory and metabolic variables were measured by

indirect calorimetry. **Results:** Altering dietary sodium or chloride had no effect on pre-exercise (baseline) pulmonary function in either group or post-exercise pulmonary function in Control subjects. However, both LSD and the $NaHCO_3$ diet improved post-exercise pulmonary function in EIA subjects compared to the NSD (which had a significantly higher chloride content compared to the other diets). In EIA subjects, comparing post- to -pre exercise, forced expiratory volume in 1-second (FEV_1) decreased by $7 \pm 4\%$ on the LSD, decreased by $14 \pm 4\%$ on the $NaHCO_3$ diet, and decreased $19 \pm 2\%$ on the NSD at 15-min post exercise. Thus there was a gradation of response from LSD, to $NaHCO_3$ diet, to NSD with respect to post-exercise pulmonary function in EIA subjects. Similar patterns were observed for forced vital capacity, forced expiratory flow rate at 25-75% FVC and peak expiratory flow rate. No changes were observed during exercise for the ventilatory and metabolic variables on the different diets for both groups. **Conclusion:** While the LSD (low sodium, low chloride) did not normalize post-exercise pulmonary function in the EIA subjects it did improve it. Likewise the $NaHCO_3$ (high sodium, low chloride) diet improved post-exercise pulmonary function, but not to the extent of the LSD. In conclusion, lowering dietary NaCl improved symptoms of EIA. Additionally, chloride is a major contributor to this response. It appears that the presence of high sodium in the diet ($NaHCO_3$) prevents the total improvement seen with the low sodium, low chloride diet (LSD).

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Introduction

It was demonstrated in the previous study (Chapter III) that a high salt diet (HSD) worsened, and a low salt diet (LSD) improved, post-exercise pulmonary function in subjects with exercise-induced asthma (EIA). The focus of previous studies has been directed at elucidating a possible mechanism, whereby the sodium ion in dietary salt (NaCl), influences airway reactivity in asthmatics (40, 55). However, it is possible that the chloride component of dietary NaCl plays a significant role in the severity of asthma and EIA.

While the mechanism by which changes in dietary NaCl may alter the severity of EIA is unknown, previous studies linking dietary NaCl with airway reactivity changes in asthma have implicated transmembrane sodium ion transport in bronchial (8, 10, 17, 55) and vascular smooth muscle contraction (16). Additionally, the influence of dietary NaCl loading could also exert a significant effect on circulating blood volume and hence airway diameter (15, 29).

In hypertension research, there have been many studies implicating the chloride ion as the main contributor to elevated blood pressure during dietary NaCl loading. Sodium loading with anions other than chloride have failed to produce elevated blood pressures in models of salt-sensitive hypertension (23, 54). In addition, several studies of NaCl-induced hypertension have suggested that the anion in NaCl may be responsible for changes in renal acid-base balance (2, 51, 52). It has been shown that a relative metabolic acidosis is present in NaCl-sensitive subjects during dietary NaCl loading, resulting in a decrease in intracellular pH ($[pH]_i$) (2, 51, 52). This association between metabolic acidosis and hypertension may occur at the level of cells other than

kidney cells, such as smooth muscle cells and mast cells, which are important in the pathogenesis of EIA. It is possible that manipulation of dietary sodium salts, with or without chloride, may have differing effects on other known or possible determinants of EIA, such as plasma membrane ion transport exchange systems and eicosanoid synthesis from mediator releasing cells in the airways.

Elucidating a role for the effect of dietary chloride on the severity of EIA has not previously been investigated. Therefore the purpose of this study was to determine the influence of high and low chloride diets on post-exercise bronchoconstriction in subjects with EIA. The experimental hypothesis tested in the present study was that both a low sodium, low chloride and a high sodium, low chloride diet would improve post-exercise pulmonary function in EIA subjects compared to a normal NaCl diet, but have no effect on post-exercise pulmonary function in Control (non-EIA) subjects.

Methods

The present study was conducted in a the same manner, and followed an identical protocol, as the previous study. Eight clinically diagnosed EIA subjects and eight subjects with no history or signs of EIA (Control), volunteered for this study, which was conducted as a double-blind crossover trial over five consecutive weeks. All subjects entered the study on their normal NaCl diet, after which they were randomly assigned to either a LSD (low sodium, low chloride) or $NaHCO_3$ (high sodium, low chloride) diet for two weeks. Thereafter, they followed a one-week washout period (NSD) and then switched to the alternative diet. Throughout the study all subjects were placed on a menu-driven diet designed to provide 1500 mg of sodium and 2315 mg of

chloride per day, whether on the LSD or $NaCHO_3$ diet. During the LSD, subjects consumed the base diet plus placebo (sucrose) capsules. However, for the $NaCHO_3$ diet, the base diet was supplemented with 14 one-gram salt capsules, which equaled 4000 mg per day of sodium and 14,370 mg per day of bicarbonate. The $NaHCO_3$ diet had the same equimolar concentration of sodium as the HSD in the previous study.

The analysis of expired gases during exercise was accomplished by open circuit spirometry, using the mixing chamber mode. Pulmonary function tests were performed at screening, and at the end of each treatment period (pre-exercise and and at 1, 5, 10, and 15-minutes post-exercise). Twenty-four urine was measured at the end of each treatment period and analyzed for sodium, chloride, potassium, and creatinine.

Statistcial Analysis. Data were analyzed using the SigmaStat 2.03 statistical package and SYSTAT 8.0 (SPSS Inc., Chicago, IL). Pre-exercise and post-exercise pulmonary function values and metabolic and ventilatory data were examined for the effect of diet (LSD, NSD, $NaHCO_3$) and the presence of EIA by a 2-factor repeated measures ANOVA, with both treatment and time as "within-subject" effects. A Tukey's post-hoc multiple pairwise comparison was used to isolate the differences ($p<0.05$). When the F-ratio was significant, an omega-squared (ω^2) was used to assess the magnitude of the treatment effect (i.e., what percent varience is accounted for by the treatments). Power was calculated at 0.925, using a sample size of $n = 8$ (per group). The data were analyzed for the presence of carry-over effects between treatments, by employing a 2×2 ANOVA crossover design (18). All statistical tests of significance were set at $p<0.05$. Data are expressed as mean $\pm$ SEM.

Results

Table IV-1 indicates that the two groups (EIA and Control) were well matched according to age and physical characteristics. However, the Control group demonstrated a significantly higher ($p<0.05$) fitness level ($VO2_{peak}$) compared to the EIA group.

Table IV-2 shows that the subjects were not using any "long-term" medications, but were using traditional short-acting or "rescue" medications. Three Control subjects did not complete the study. However, three additional Control subjects were recruited and did complete the study. All EIA subjects tested positive for EIA during the initial screening test, while none of the Control subjects tested positive for EIA. Subjects demonstrated dietary compliance with sodium enhancement on the $NaHCO_3$ diet and sodium restriction on the LSD as indicated by the 24-hr urinary excretion of sodium (Table IV-3). Additionally, there was no significant difference between chloride excretions on the LSD and $NaHCO_3$ diet (Table IV-3). Neither potassium nor creatinine excretions were altered by the diets (Table IV-3).

No significant differences were observed for pre-exercise (baseline) pulmonary function on each diet in either group (Table IV-4). All baseline pulmonary function values for the three diets fell within the normal parameters established for males and females at rest (11), indicating that no airflow limitations were present.

Table IV-5 shows the post-exercise pulmonary values for the Control subjects. There were no significant differences in post-exercise pulmonary values by time or diet. However, for the EIA subjects, the post-exercise values (Table IV-6) for FVC, FEV_1, $FEF_{25-75\%}$ and PEFR were highest for LSD and lowest for the NSD. The $NaHCO_3$ diet was

intermediate between the LSD and NSD. There was a significant difference between the LSD and NSD ($p<0.05$), but not between the $NaHCO_3$ diet and the LSD ($p>0.05$), or NSD ($p>0.05$) for post-exercise pulmonary function in EIA subjects. The FEV_1/FVC ratio decreased on all diets post-exercise compared to pre-exercise values. However, the FEV_1/FVC ratio was not altered between diets post-exercise.

Figures IV-1 and IV-5 demonstrate the differential effect of the percent change in FEV_1 pre- to -post exercise for Control and EIA subjects respectively. No significant differences ($p>0.05$) in the percent change in FEV_1 (Table IV-1), FVC (Table IV-2), $FEF_{25-75\%}$ (Table IV-3), and PEFR (Table IV-4) pre- to post-exercise were observed for the Control subjects on any diet. The EIA subjects demonstrated a significant reduction ($p<0.05$) in the percent change in FEV_1 pre- to -post exercise (Table IV-5), in a graded fashion from LSD ($7.0 \pm 4\%$) to $NaHCO_3$ diet ($14.0 \pm 4\%$) to NSD ($19.0 \pm 2\%$) at 15-min post-exercise. In addition, EIA subjects demonstrated significant reductions in FVC (Table IV-6), $FEF_{25-75\%}$ (Table IV-7) and PEFR (Table IV-8) pre- to post-exercise, in a graded response from LSD to NSD to HSD, by time for each of the diet periods.

Pre- and post-exercise pulmonary function values at 1, 5, 10 and 15 minutes are presented for Control subjects in Figures IV-9 to IV-12. The Control subjects demonstrated no significant differences ($p>0.05$) by time for each of the diet periods. However, the EIA subjects (Figures IV-13 to IV-16) demonstrated significant differences ($p<0.05$) in all the pulmonary function variables measured at 5, 10, and 15 minutes post-exercise between the LSD and NSD. The $NaHCO_3$ diet elicited no significant difference ($p>0.05$) between the LSD and NSD by time, for any pulmonary function variable measured post-exercise.

An ω^2 was performed on pulmonary function values for EIA subjects at 15 minutes post-exercise and revealed that 87% (FEV_1), 88% (FVC), 53% ($FEF_{25-75\%}$) and 68% (PEFR) of the variance was accounted for by the treatments.

Ventilatory and metabolic variables measured during exercise for Control and EIA subjects are presented in Tables IV-7 to IV-11 and Tables IV-12 to IV-16 respectively. Both Control and EIA subjects demonstrated no significant differences in any of the variables measured during exercise by diet.

Because exercise ventilation plays a role in triggering post-exercise bronchoconstriction (1, 5, 28), regression analysis was used to examine the effect of ventilation on the pre- to -post exercise change in $FEV_1$for Control (Figures IV-17) and EIA subjects (Figures IV-18). It is apparent that maximal exercise ventilation did not correlate with decrements in pulmonary function observed on the three diets in Control and EIA subjects. In fact, for EIA subjects, the trend is just the opposite, exercise ventilations correspond with improved FEV_1 values. Therefore, post-exercise pulmonary function changes are a function of diet and not maximal exercise ventilation.

A 2 x 2 ANOVA crossover design was used to test for the presence of carry-over effects and indicated that none were present ($p>0.05$) for all measures of lung function. An ANOVA for 2 x 2 crossover design for EIA subjects at FEV_1 at 10 minutes post-exercise is shown in Table IV-17. Carry-over effects and period effects were not significant ($p>0.05$). Figures IV-25 to IV-28 provide visual confirmation that carry-over effects were not significant and that there is no group-by-period interaction. Carry-over effects assessed for FEV_1 at 1, 5, and 15-minutes post-exercise, and for FVC, $FEF_{25-75\%}$, and PEFR at 1, 5, 10, 15-minutes post exercise were not significant in EIA subjects. In

addition, carry-over effects were not significant for Control subjects for all post-exercise pulmonary function measures at 1, 5, 10, and 15-minutes post-exercise.

Discussion

The previous study (Chapter III) demonstrated that manipulation of dietary NaCl effects post-exercise pulmonary function in subjects with EIA. The results of the present study confirms this finding. Additionally, the current data indicates that dietary chloride manipulation alters post-exercise pulmonary function in subjects with EIA. The current study represents the first report that high sodium loading with low chloride ($NaHCO_3$) improves pulmonary function in EIA subjects, but not to the extent of the LSD (low sodium, low chloride). This implicates the chloride ion in the effect of dietary NaCl on the severity of EIA. However, it appears that the sodium ion also plays an important role, as high sodium in the $NaHCO_3$ diet prevented the total improvement seen with a LSD.

Dietary goals were met and subject compliance was successful in the current study as, compared to the NSD, sodium decreased significantly while on the LSD and increased significantly while on the $NaHCO_3$ diet. Thus, a graded dose of dietary sodium was achieved in this study from 1, 466 – 1, 761 mg/day (LSD) to 6, 260 – 6, 266 mg/day (HSD). In addition, dietary chloride goals were met as there was no significant difference between the LSD (2, 805 – 3, 043 mg/day) and $NaHCO_3$ (2, 722 – 2, 794 mg/day); both were significantly lower than the NSD (4, 442 – 4, 699 mg/day). There was no change in potassium excretion or in glomerular filtration rate, as indicated by creatinine excretion.

In the present study, the FVC maneuver was used to evaluate the integrity of the caliber of the airways pre- and -post exercise on various sodium salt diets in EIA and Control subjects. Pre- to -post-exercise changes were evaluated in both groups. Post-exercise pulmonary function in EIA subject improved on the LSD and worsened on the NSD, with the $NaHCO_3$ being intermediate, suggesting less airway obstruction on the LSD and $NaHCO_3$, compared to the NSD. Control subjects demonstrated no differences for these values with respect to the different sodium diets.

The pattern of exercise ventilation during exercise did not differ with diet in the EIA subjects. This is suprising, since in the previous study there was significant differences noted between the LSD and HSD for V_T and f_b selection, with the HSD resulting in a higher V_T and lower f_b selection, while the LSD reversed this pattern, lower V_T and higher f_b. This pattern of breathing indicates increased airways resistance on the HSD and decreased airways resistance on the LSD. The HSD in the previous study and the $NaHCO_3$ diet in the current study contained the same equimolar amount of sodium. This suggests that the chloride ion may also be involved in the pulmonary function response during exercise in EIA subjects. Further studies are needed to elucidate the role of dietary chloride on airway function during exercise in EIA subjects.

The biological mechanisms that might explain the role of dietary salt intake in airway responsiveness remain uncertain. It has been shown that high dietary sodium loads inhibit Na^+/K^+ ATPase activity in erythrocytes of normotensive males (57). The mechanism of this inhibition is unclear. However, with increasead sodium influx and an inhibited Na^+/K^+ ATPase, $Na^+ - Ca^{2+}$ exchange could become the predominant mechanism for restoring intracellular sodium ($[Na^+]_i$) levels toward normal. This in turn

could lead to a rise in free intracellular calcium ($[Ca^{2+}]_i$), and an increase in bronchial smooth muscle contraction (10). However, it is possible that the chloride ion may also play a significant role in mediating the severity of EIA. Medici et al. (32) substituted NaCl with sodium citrate in an equimolar concentration in 14 asthmatics. They demonstrated that while bronchial reactivity was sensitive to dietary changes in NaCl, this effect was most likely mediated by the sodium ion, and not the chloride ion.

Although the mechanism by which dietary NaCl influences the severity of EIA is unknown it could be due to intrinsic alterations of cellular ion transport. A common etiological role of dietary NaCl to both asthma and hypertension has been suggested (7). Increasing evidence has suggested that the anionic component of NaCl may contribute to hypertension in several animal models (20, 23, 39, 60). Kurtz et al. (23) and others (24, 39, 58) using rats given desoxycorticosterone (DOC), observed that NaCl induced hypertension, while an equimolar amount of sodium in dietary $NaHCO_3$ did not. They found that replacing dietary chloride with bicarbonate, without changing dietary sodium, reversed hypertension induced by DOC and NaCl. These findings suggest that, in rats given DOC and a fixed amount of dietary sodium, the anionic component of the sodium salt can determine the occurrence, progression, and reversal of hypertension.

Kurtz et al. (21, 22) extended their observations to humans by substituting sodium citrate for NaCl in the diets of salt-sensitive hypertensive men. They demonstrated that NaCl elevated blood pressure while sodium citrate did not. Shore et al. (54) performed a similar study in 6 men with essential hypertension by substituting sodium phosphate for sodium citrate. Blood pressure increased on NaCl supplementation, but not on the sodium phosphate. Luft et al. (27) tested the hypothesis

that NaCl and $NaHCO_3$ have opposite effects on blood pressure in 10 mildly hypertensive men. They showed that the addition of $NaCHO_3$ significantly lowered systolic blood pressure while NaCl did not increase blood pressure. The data are supported by the results of Morgan (35), that changes in blood pressure depend on dietary chloride content.

Numerous studies of NaCl-induced hypertension have shown that the anion may be responsible for changes in renal-acid base homeostasis (2, 51, 52). These studies have shown that sodium salts such as $NaHCO_3$ and sodium citrate induce a metabolic alkalosis and do not raise blood pressure as effectively as NaCl in hypertensive individuals, and suggests a role for the anion in the pressor effect of NaCl. It has been demonstrated (2, 51, 52) that a relative metabolic acidosis is present in NaCl-sensitive subjects during dietary NaCl loading with an attendant decrease in $[pH]_i$. This link between metabolic acidosis and hypertension can be envisioned at the level of cells other than kidney cells, such as smooth muscle cells and mast cells, which may be important in the pathogenesis of acute bronchospasm in EIA. Manipulation of dietary sodium salts, with or without chloride, may have different effects on other known or possible determinants of EIA, such as eicosanoid synthesis, sympathetic nervous system activity, and the plasma membrane Na^+/H^+ antiporter mechanism.

In lymphocytes obtained from a rat model of genetic hypertension, it has been demonstrated that increased metabolic acid production is compatible with intracellular acidosis, where reduced $[pH]_i$ stimulates the Na^+/H^+ exchange mechanism, thereby promoting Na^+ cellular influx and increasing intracellular Na^+ ($[Na^+]_i$) concentrations (2, 3, 12, 14, 37). This rise in $[Na^+]_i$ may contribute to an increase in $[Ca^{2+}]_i$ via the Na^+/Ca^{2+}

exchange mechanism (2, 3). In addition, $[Ca^{2+}]_i$ may also be increased via Ca^{2+}/H^+ exchange (2, 3). It has been shown that provision of sodium as $NaHCO_3$ or sodium citrate can suppress the overactivity of the Na^+/H^+ antiporter by neutralizing acid overproduction generated during NaCl loading (2, 4). In response to a metabolic acidosis the activity of the N^+/H^+ antiporter has been shown to increase in renal proximal cells (42, 50, 56), platelets (26, 49), erythrocytes (45) and leukocytes (2, 3, 44) of individuals with essential hypertension and in the salt-sensitive Dahl/Rapp rat. Although extrapolation of data from cells other than bronchial smooth muscle cells to the pathogenesis of EIA is speculative, it is possible that a reduced $[pH]_i$, resulting in increased $[Ca^{2+}]_i$ also occurs in bronchial smooth muscle cells, leading to increased contractility.

It has been proposed that $[pH]_i$ regulation is governed by three plasma membrane pH_i regulatory proteins; Na^+/H^+ antiporter, Na^+-dependent Cl^-/HCO_3^- exchanger, and Na^+-independent Cl^-/HCO_3^- exchanger) (2, 3, 44, 48). Clearly, if $[pH]_i$ or the Na^+/H^+ antiporter is to be implicated in the pathogenesis of EIA, then HCO_3^- - dependent mechanisms cannot be ignored. The tendency toward intracellular acidosis should enhance not only Na^+/H^+ exchange, but Na^+ -dependent Cl^-/HCO_3^- exchange as well (2), which defends against cell acidification by acting as a pathway for HCO_3^- entry into the cell in exchange for intracellular Cl^- (2-4). Both transporters thus regulate pH_i and Na^+ entry into the cell. Batlle et al. (2, 3) and Redon et al. (44), studied the activity of both the Na^+ -dependent and Na^+ -independent Cl^-/HCO_3^- exchangers in lymphocytes from rats with genetic hypertension. They demonstrated that change in the pH_i was clearly dependent on extracellular Cl^- concentrations, such that an increase

in external Cl^- resulted in increased activity of the Na^+ -independent Cl^-/HCO_3^- exchanger, which exchanges extracellular Cl^- for intracellular HCO_3^-, resulting in a concomitant fall in $[pH]_i$.

Ionic fluxes that contribute to changes in membrane potential and variations in pH_i are not well known in eosinophils, basophils or mast cells, although they may be important in the stimulus-response coupling cascade. Mast cells are important in the pathogenesis of acute bronchospasm following exercise due to activation of pro-inflammatory mediator release, such as histamine, prostaglandins and leukotrienes. It has been shown that mast cells contain an N^+/H^+ antiport, Na^+/K^+ ATPase, and an Cl^-/HCO_3^- antiport (9, 13, 19, 34, 41, 43, 47).

It is conceivable that a high NaCl diet may mediate the release of eicosanoids (prostaglandins, sulphidopeptide leukotrienes and thromboxane) from cells in the airways, such as mast cells, eosinophils, granulocytes, basophils, macrophages, and epithelial cells (31, 59). There is considerable evidence that eicosanoids play a role in the pathogenesis of EIA. It has been shown that manipulation of dietary NaCl has a direct effect on prostaglandin synthesis, with respect to renal sodium balance and vascular smooth muscle (25), and it has been suggested that a high NaCl diet suppresses PGE_2, a bronchodilator (31, 59). It has also been demonstrated in rat peritoneal mast cells that a high $[Na^+]_i$ increases $[Ca^{2+}]_i$, via inhibition of the Na^+/Ca^{2+} exchanger, resulting in histamine release (41). The effects of dietary NaCl loading on the role of eicosanoids and histamine from mast cells in subjects with EIA remain to be determined.

Metabolic acidosis is a common finding in patients with severe acute asthma. This acidosis is not due to lactic acidosis in most cases, but is probably due to renal

bicarbonate (HCO_3) loss (6, 30, 36, 38). Interestingly, infusions of $NaHCO_3$ have been used to alleviate severe bronchospasms in patients with acute bronchial asthma (33, 46, 53). Sodium bicarbonate administration could induce a metabolic alkalosis by raising the HCO_3^-/PCO_2 ratio and thus the pH, decrease the H^+ stimulation of carotid bodies, and thereby raise the regulated level of arterial PCO_2 and decrease ventilation at a given level of exercise (respiratory compensation). However in the present study, $NaHCO_3$ loading failed to decrease exercise V_E compared to the NSD in both the Control and EIA subjects. Since there was no change in exercise V_E between treatments in EIA subjects, and since blood gas analysis was not performed (in order to determine plasma pH and PCO_2), it is difficult to interpret whether the reduction in the severity of EIA seen with $NaHCO_3$ loading compared to the NSD, is due to low chloride in the diet, or HCO_3^- loading. Whatever the mechanism, the physiological role that HCO_3 loading may play in this improvement cannot be discounted.

In conclusion, while the mechanism is imprecise regarding changes in dietary NaCl's effect on post-exercise pulmonary function in EIA, it is apparent that in the current study the $NaHCO_3$ diet improved post-exercise pulmonary function in the presence of high sodium, but not to the extent of the LSD. The lowering of dietary NaCl did improve symptoms of EIA, but did not normalize pulmonary function. Additionally, chloride may be a major contributor to this response. The presence of high sodium in the $NaHCO_3$ diet prevents the total improvement seen with combined low sodium, low chloride (LSD). An alternative interpretation of these results with regards to the potential effect of HCO_3^- per se on EIA cannot be ignored.

References

1. Anderson, S. D., and E. Daviskas. The airway microvasculature and exercise i nduced asthma. *Thorax* 47: 748-52, 1992.

2. Batlle, D., J. Redon, C. Gutterman, M. LaPointe, A. Saleh, A. Sharma, G. Rombola, M. Ye, W. Alsheikha, L. Gomez, and et al. Acid-base status and intracellular pH regulation in lymphocytes from rats with genetic hypertension. *J Am Soc Nephrol* 5: S12-22, 1994.

3. Batlle, D. C., A. Saleh, and G. Rombola. Reduced intracellular pH in lymphocytes from the spontaneously hypetensive rat. *Hypertension* 15: 97-103, 1990.

4. Batlle, D. C., A. M. Sharma, M. W. Alsheikha, M. Sobrero, A. Saleh, and C. Gutterman. Renal acid excretion and intracellular pH in salt-sensitive genetic hypertension. *J Clin Invest* 91: 2178-84, 1993.

5. Beck, K. C. Control of airway function during and after exercise in asthmatics. *Med Sci Sports Exerc* 31: S4-11, 1999.

6. Bouachour, G., P. Tirot, N. Varache, P. Harry, and P. Alquier. [Metabolic acidosis in severe acute asthma. Effect of alkaline therapy]. *Rev Pneumol Clin* 48: 115-9, 1992.

7. Burney, P. A diet rich in sodium may potentiate asthma. Epidemiologic evidence for a new hypothesis. *Chest* 91: 143S-148S, 1987.

8. Burney, P. G., J. E. Neild, C. H. Twort, S. Chinn, T. D. Jones, W. D. Mitchell, C. Bateman, and I. R. Cameron. Effect of changing dietary sodium on the airway response to histamine. *Thorax* 44: 36-41, 1989.

9. Cabado, A. G., M. R. Vieytes, and L. M. Botana. Inhibition of Na+/K+ ATPase under hypertonic conditions in rat mast cells. *Life Sci* 63: 1227-37, 1998.

10. Carey, O. J., C. Locke, and J. B. Cookson. Effect of alterations of dietary sodium on the severity of asthma in men. *Thorax* 48: 714-8, 1993.

11. Crapo, R. O., A. H. Morris, and R. M. Gardner. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 123: 659-64, 1981.

12. Feig, P. U., M. A. D'Occhio, and J. W. Boylan. Lymphocyte membrane sodium-proton exchange in spontaneously hypertensive rats. *Hypertension* 9: 282-8, 1987.

13. Friis, U. G., and T. Johansen. Dual regulation of the Na+/H+ -exchange in rat peritoneal mast cells: role of protein kinase C calcium on pHi regulation and histamine release. *Brit J Pharmacol* 118: 1327-1334, 1996.

14. Goldsmith, D. J., R. M. Tribe, L. Poston, F. P. Cappuccio, N. D. Markandu, G. A. MacGregor, and P. J. Hilton. Leucocyte intracellular pH and Na(+)-H+ exchange activity in essential hypertension: an in vitro study under physiological conditions. *J Hypertens* 9: 645-53, 1991.

15. Hogg, J. C., P. D. Pare, and R. Moreno. The effect of submucosal edema on airways resistance. *Am Rev Respir Dis* 135: S54-6, 1987.

16. Ives, H. E. Ion transport defects and hypertension. Where is the link? *Hypertension* 14: 590-7, 1989.

17. Javaid, A., M. J. Cushley, and M. F. Bone. Effect of dietary salt on bronchial reactivity to histamine in asthma. *Br Med J* 297: 454, 1988.

18. Jones, B., and M. G. Kenward. The 2 x 2 cross-over trial with continuous data. In: *Design and Analysis of Cross-over trials.* London: Chapman and Hall, 1989, p. 16-88.

19. Knudsen, T. The Na+/K(+)-pump in rat peritoneal mast cells: some aspects of regulation of activity and cellular function. *Dan Med Bull* 42: 441-54, 1995.

20. Kotchen, T. A., R. G. Luke, C. E. Ott, J. H. Galla, and S. Whitescarver. Effect of chloride on renin and blood pressure responses to sodium chloride. *Ann Intern Med* 98: 817-22, 1983.

21. Kurtz, T. W. Dietary chloride as a possible determinant of NaCl - sensitive hypertension (HTN) in man. *Kid Intern* 31: A301, 1987.

22. Kurtz, T. W., H. A. Al-Bander, and R. C. Morris, Jr. "Salt-sensitive" essential hypertension in men. Is the sodium ion alone important? *N Engl J Med* 317: 1043-8, 1987.

23. Kurtz, T. W., and R. C. Morris. Dietary chloride and bicarbonate as determinants of desoxycortocosterone hypertension. *J Hypertens* 2: 371-373, 1984.

24. Kurtz, T. W., and R. C. Morris, Jr. Dietary chloride as a determinant of "sodium-dependent" hypertension. *Science* 222: 1139-41, 1983.

25. Limas, C., P. Goldman, C. J. Limas, and J. Iwai. Effect of salt on prostaglandin metabolism in hypertension-prone and - resistant Dahl rats. *Hypertension* 3: 219-24, 1981.

26. Livne, A., J. W. Balfe, R. Veitch, A. Marquez-Julio, S. Grinstein, and A. Rothstein. Increased platelet Na+-H+ exchange rates in essential hypertension: application of a novel test. *Lancet* 1: 533-6, 1987.

27. Luft, F. C., M. B. Zemel, J. A. Sowers, N. S. Fineberg, and M. H. Weinberger. Sodium bicarbonate and sodium chloride: effects on blood pressure and electrolyte homeostasis in normal and hypertensive man. *J Hypertens* 8: 663-70, 1990.

28. Mahler, D. A. Exercise-induced asthma. *Med Sci Sports Exerc* 25: 554-61, 1993.

29. McFadden, E. R., Jr. Hypothesis: exercise-induced asthma as a vascular henomenon [see comments]. *Lancet* 335: 880-3, 1990.

30. McFadden, E. R., Jr., and H. A. Lyons. Arterial-blood gas tension in asthma. *N Engl J Med* 278: 1027-32, 1968.

31. McGiff, J. C., and J. R. Vane. Prostaglandins and the regulation of blood pressure. *Kidney Int Suppl*: S262-70, 1975.

32. Medici, T. C., A. Z. Schmid, M. Hacki, and W. Vetter. Are asthmatics salt-sensitive? A preliminary controlled study. *Chest* 104: 1138-43, 1993.

33. Mentilove, S. M., and R. A. Goldring. Combined ventilator and bicarbonate strategy in the management of status asthmaticus. *Am J Med* 74: 898-901, 1983.

34. Meyer, G., S. Doppierio, P. Vallin, and L. Daffonchio. Effect of frusemide on Cl-channel in rat peritoneal mast cells. *Eur Respir J* 9: 2461-7, 1996.

35. Morgan, T. O. The effect of potassium and bicarbonate ions on the rise in blood pressure caused by sodium chloride. *Clinical Science* 63: 407S-409S, 1982.

36. Mountain, R. D., J. E. Heffner, N. C. Brackett, Jr., and S. A. Sahn. Acid-base disturbances in acute asthma. *Chest* 98: 651-5, 1990.

37. Ng, L. L., D. A. Fennell, and C. Dudley. Kinetics of the human leucocyte Na(+)-H+ antiport in essential hypertension. *J Hypertens* 8: 533-7, 1990.

38. Okrent, D. G., S. Tessler, R. A. Twersky, and D. P. Tashkin. Metabolic acidosis not due to lactic acidosis in patients with severe acute asthma. *Crit Care Med* 15: 1098-101, 1987.

39. Passmore, J. C., S. A. Whitescarver, C. E. Ott, and T. A. Kotchen. Importance of chloride for deoxycorticosterone acetate-salt hypertension in the rat. *Hypertension* 7: I115-20, 1985.

40. Pavord, I. D., S. Dutt, O. J. Carey, D. Spence, S. Brand, and R. F. Bing. Red blood cell sodium transport in asthma. *Thorax* 45: 799, 1990.

41. Praetorius, H. A., U. G. Friis, J. Praetorius, and T. Johansen. Evidence for a Na+/Ca2+ exchange mechanism in rat peritoneal mast cells. *Pflug Arch* 437: 86-93, 1998.

42. Preisig, P. A., and R. J. Alpern. Chronic metabolic acidosis causes an adaptation in the apical membrane Na/H antiporter and basolateral membrane Na(HCO3)3 symporter in the rat proximal convoluted tubule. *J Clin Invest* 82: 1445-53, 1988.

43. Ramirez-Gil, J. F., P. Trouve, N. Mougenot, A. Carayon, P. Lechat, and D. Charlemagne. Modifications of myocardial Na+,K(+)-ATPase isoforms and Na+/Ca2+ exchanger in aldosterone/salt-induced hypertension in guinea pigs. *Cardiovasc Res* 38: 451-62, 1998.

44. Redon, J., and D. Batlle. Regulation of intracellular pH in the spontaneously hypertensive rat. Role of bicarbonate-dependent transporters. *Hypertension* 23: 503-12, 1994.

45. Resnick, L. M., R. K. Gupta, R. E. Sosa, M. L. Corbett, and J. H. Laragh. Intracellular pH in human and experimental hypertension. *Proc Natl Acad Sci* 84: 7663-7, 1987.

46. Roncoroni, A. J., H. J. Adrougue, C. W. De Obrutsky, M. L. Marchisio, and M. R. Herrera. Metabolic acidosis in status asthmaticus. *Respiration* 33: 85-94, 1976.

47. Rumpel, E., U. Pilatus, A. Mayer, and I. Pecht. Na+ and Ca2+ gradients across the membrane modulate the secretory response of mast cells. *Int Arch Allergy Immunol* 107: 351-3, 1995.

48. Saleh, A. M., and D. C. Batlle. Kinetic properties of the Na+/H+ antiporter of lymphocytes from the spontaneously hypertensive rat: role of intracellular pH. *J Clin Invest* 85: 1734-39, 1990.

49. Schmouder, R. L., and A. B. Weder. Platelet sodium-proton exchange is increased in essential hypertension. *J Hypertens* 7: 325-30, 1989.

50. Sharma, A. M., C. Cetto, U. Schorr, K. P. Spies, and A. Distler. Renal acid-base excretion in normotensive salt-sensitive humans. *Hypertension* 22: 884-90, 1993.

51. Sharma, A. M., and A. Distler. Acid-base abnormalities in hypertension. *Am J Med Sci* 307 Suppl 1: S112-5, 1994.

52. Sharma, A. M., A. Kribben, S. Schattenfroh, C. Cetto, and A. Distler. Salt sensitivity in humans is associated with abnormal acid-base regulation. *Hypertension* 16: 407-13, 1990.

53. Shires, R., B. I. Joffe, L. G. Heding, and H. C. Seftel. Metabolic studies in acute asthma before and after treatment. *Br J Dis Chest* 73: 66-70, 1979.

54. Shore, A. C., N. D. Markandu, and G. A. MacGregor. A randomized crossover study to compare the blood pressure response to sodium loading with and without chloride in patients with essential hypertension [published erratum appears in J Hypertens 1988 Nov;6(11):i]. *J Hypertens* 6: 613-7, 1988.

55. Tribe, R. M., J. R. Barton, L. Poston, and P. G. Burney. Dietary sodium intake, airway responsiveness, and cellular sodium transport. *Am J Respir Crit Care Med* 149: 1426-33, 1994.

56. Warnock, D. G., and J. Eveloff. NaCl entry mechanisms in the luminal membrane of the renal tubule. *Am J Physiol* 242: F561-74, 1982.

57. Weissberg, P. L., M. J. West, M. J. Kendall, M. Ingram, and K. L. Woods. Effect of changes in dietary sodium and potassium on blood pressure and cellular electrolyte handling in young normotensive subjects. *J Hypertens* 3: 475-80, 1985.

58. Whitescarver, S. A., B. J. Holtzclaw, J. H. Downs, C. E. Ott, J. R. Sowers, and T. A. Kotchen. Effect of dietary chloride on salt-sensitive and renin-dependent hypertension. *Hypertension* 8: 56-61, 1986.

59. Whitescarver, S. A., C. E. Ott, B. A. Jackson, G. P. Guthrie, and T. A. Kotchen. Salt-sensitive hypertension: contribution of chloride. *Science* 223: 1430-32, 1984.

60. Wyss, J. M., M. Liumsiricharoen, W. Sripairojthikoon, D. Brown, R. Gist, and S. Oparil. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension* 9: III171-5, 1987.

Table IV-1. Subject characteristics.

Group	Gender M/F	Age (years)	Weight (kg)	Height (cm)	$VO2_{peak}$ (NSD) (ml/min/kg)
EIA	4,4	22 ± 1.5	67 ± 4	171 ± 2	42.8 ± 1.4
Control	4,4	23 ± 0.9	68 ± 2	171 ± 3	54.1 ± 1.4

Values are means ± SEM. Significant difference between VO_{2peak} for EIA and Control group, $p<0.05$.

Table IV-2 Subject medications for EIA subjects.

Subject	Medication
1	Albuterol (Proventil)
2	Albuterol (Proventil)
3	Terbutaline (Bricanyl)
4	Albuterol (Ventolin)
5	Albuterol (Proventil)
6	Terbutaline (Bricanyl)
7	Albuterol (Ventolin)
8	Terbutaline (Bricanyl)

Medications are all short-acting β_2 agonists used for rescue medication. No subjects appeared to be on drugs used for maintenance therapy.

Table IV-3. Twenty-four hour urinary excretion of sodium, potassium, chloride and creatinine (mg/day).

Group	LSD	NSD	$NaHCO_3$
EIA:			
Sodium	1,761 ± 202[a]	3,447 ± 220[b]	6,266 ± 713[c]
Chloride	3,043 ± 453[a]	4699 ± 476[b]	2722 ± 241[a]
Potassium	1,833 ± 252	2,183 ± 222	2,141 ± 182
Creatinine	1,415 ± 109	1,722 ± 272	1,426 ± 70
Sodium normalized to creatinine	1.24 ± 0.22[a]	2.00 ± 0.37[a]	4.39 ± 0.56[c]
24-hour volume (ml)	893 ± 37[a]	1,146 ± 84[b]	1,543 ± 115[c]
Control:			
Sodium	1,466 ± 146[a]	3,466 ± 222[b]	6,260 ± 339[c]
Chloride	2,805 ± 154[a]	4,442 ± 303[b]	2,794 ± 192[a]
Potassium	1,629 ± 190	1,456 ± 202	1,548 ± 188
Creatinine	1,470 ± 193	1,543 ± 200	1,656 ± 216
Sodium normalized to creatinine	1.00 ± 0.23[a]	2.24 ± 0.55[b]	3.78 ± 0.69[c]
24-hour volume (ml)	1,139 ± 139[a]	1,601 ± 167[b]	2,112 ± 204[c]

Values are means ± SEM. Letters [a,b,c] designate significance ($p<0.05$) among values, when letters are different. There are no significant differences among potassium or creatinine values ($p>0.05$).

Table IV-4. Baseline pulmonary function.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
EIA:					
LSD	5.10 ± 0.25	4.10 ± 0.24	80.4 ± 3	3.25 ± 0.15	7.70 ± 0.37
NSD	5.00 ± 0.29	4.02 ± 0.21	80.4 ± 2	3.50 ± 0.19	7.50 ± 0.58
$NaHCO_3$	4.92 ± 0.27	4.14 ± 0.23	84.1 ± 3	3.41 ± 0.12	7.43 ± 0.47
Control:					
LSD	5.51 ± 0.29	4.26 ± 0.21	77.0 ± 2	4.77 ± 0.52	8.95 ± 0.84
NSD	5.55 ± 0.32	4.42 ± 0.39	80.0 ± 3	4..89 ± 0.46	9.60 ± 0.67
$NaHCO_3$	5.56 ± 0.28	4.39 ± 0.33	80.0 ± 3	4.92 ± 0.47	9.00 ± 0.82

Values are mean ± SEM. There are no significant differences for any variables among diets or groups ($p>0.05$). FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-sec; $FEF_{25-75\%}$, forced expiratory flow rate from 25-75% of FVC; PEFR, peak expiratory flow rate. LSD, low salt diet; NSD, normal salt diet; $NaHCO_3$-, sodium bicarbonate diet.

Table IV-5. Pulmonary function post-exercise in Control subjects.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
LSD:					
1min	5.40 ± 0.26	4.25 ± 0.33	78.7 ± 3	4.94 ± 0.06	9.20 ± 0.79
5min	5.41 ± 0.28	4.24 ± 0.33	78.3 ± 3	4.74 ± 0.53	9.10 ± 0.77
10min	5.39 ± 0.32	4.24 ± 0.34	78.7 ± 2	4.68 ± 0.52	8.85 ± 0.84
15min	5.42 ± 0.32	4.18 ± 0.35	77.1 ± 3	4.67 ± 0.51	8.85 ± 0.80
NSD:					
1min	5.42 ± 0.29	4.37 ± 0.35	80.6 ± 3	5.00 ± 0.59	9.69 ± 0.73
5min	5.44 ± 0.32	4.40 ± 0.35	80.9 ± 2	4.96 ± 0.56	9.41 ± 0.71
10min	5.42 ± 0.34	4.38 ± 0.37	80.8 ± 3	4.90 ± 0.53	9.39 ± 0.75
15min	5.45 ± 0.31	4.38 ± 0.35	80.4 ± 3	4.82 ± 0.50	9.34 ± 0.73
$NaHCO_3$:					
1min	5.45 ± 0.25	4.34 ± 0.32	79.6 ± 3	5.02 ± 0.61	9.27 ± 0.82
5min	5.40 ± 0.32	4.31 ± 0.34	79.8 ± 3	4.88 ± 0.50	9.17 ± 0.80
10min	5.52 ± 0.30	4.33 ± 0.34	78.4 ± 2	4.83 ± 0.52	9.23 ± 0.71
15min	5.44 ± 0.32	4.32 ± 0.27	79.4 ± 3	4.88 ± 0.56	9.03 ± 0.77

Values are mean ± SEM. There were no significant differences in Control subjects for post-exercise values by time or diet ($p>0.05$).

Table IV-6. Pulmonary function post-exercise in EIA subjects.

	FVC (L)	FEV_1 (L)	FEV_1/ FVC (%)	$FEF_{25-75\%}$ (L/s)	PEFR (L/s)
LSD:					
1min	5.11 ± 0.26*[a]	4.17 ± 0.25*[a]	81.2 ± 3*[a]	3.38 ± 0.08*[a]	7.78 ± 0.40*[a]
5min	5.14 ± 0.30*[a]	4.00 ± 0.25*[a]	77.8 ± 4*[a]	3.12 ± 0.06*[a]	7.43 ± 0.35*[a]
10min	4.83 ± 0.31*[a]	3.84 ± 0.23*[a]	79.5 ± 3*[a]	3.02 ± 0.07*[a]	6.72 ± 0.46*[a]
15min	4.69 ± 0.31*[a]	3.78 ± 0.23*[a]	80.6 ± 3*[a]	2.79 ± 0.11*[a]	6.54 ± 0.49*[a]
NSD:					
1min	4.80 ± 0.30*[a]	3.78 ± 0.26*[a]	78.7 ± 2*[a]	3.15 ± 0.22*[a]	7.11 ± 0.58*[a]
5min	4.63 ± 0.31*[b]	3.47 ± 0.25*[b]	74.9 ± 3*[a]	2.70 ± 0.25*[a]	6.25 ± 0.64*[b]
10min	4.40 ± 0.31*[b]	3.40 ± 0.24*[b]	77.3 ± 3*[a]	2.37 ± 0.26*[b]	5.80 ± 0.65*[b]
15min	4.19 ± 0.31*[b]	3.23 ± 0.22*[b]	77.0 ± 2*[a]	2.13 ± 0.27*[b]	5.31 ± 0.69*[b]
$NaHCO_3$:					
1min	4.96 ± 0.28*[a]	4.10 ± 0.25*[a]	82.7 ± 3*[a]	3.27 ± 0.12*[a]	7.44 ± 0.49*[a]
5min	4.87 ± 0.31*[a,b]	3.90 ± 0.25*[a,b]	80.0 ± 3*[a]	2.86 ± 0.21*[a]	6.83 ± 0.50*[a,b]
10min	4.57 ± 0.31*[a,b]	3.62 ± 0.28*[a,b]	79.2 ± 3*[a]	2.60 ± 0.23*[a,b]	6.26 ± 0.57*[a,b]
15min	4.56 ± 0.31*[a]	3.57 ± 0.27*[a,b]	78.3 ± 2*[a]	2.39 ± 0.23*[a,b]	5.90 ± 0.65*[a]

Values are mean ± SEM. *$p<0.05$ compared to respective pre-exercise value. Letters [a,b,c] refer to comparisons by diet for the post-exercise time period within specific variable; different letters designate significant difference ($p<0.05$). Values with the same letter are not statistically significant ($p>0.05$).

Table IV-7. Cardiovascular changes occurring during exercise in Control subjects.

	LSD	NSD	$NaHCO_3$
HR (bpm)			
Rest	83 ± 5.7	84 ± 7.9	83 ± 5.0
Steady state exercise	161 ± 1.2	161 ± 1.6	158 ± 1.8
Peak Exercise	185 ± 6.7	181 ± 9.9	173 ± 9.8
HR %predicted max (bpm)			
Rest	47 ± 2.8	45 ± 3.9	45 ± 2.6
Steady state exercise	85 ± 0.5	88 ± 1.3	85 ± 0.4
Peak exercise	99 ± 3.1	97 ± 5.0	93 ± 5.0
O_2 Pulse (ml/beat)			
Rest	3.6 ± 0.5	3.5 ± 0.3	3.4 ± 0.4
Steady state exercise	17.9 ± 1.0	18.4 ± 1.0	17.4 ± 1.3
Peak Exercise	21.2 ± 1.3	20.8 ± 1.9	20.4 ± 1.6

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p>0.05$. HR, heart rate; HR%, HR as a percentage of maximum heart rate (220-Age); O_2 Pulse, oxygen pulse (VO_2/HR).

Table IV-8. Breathing pattern and exercise ventilation occurring during exercise in Control subjects.

	LSD	NSD	$NaHCO_3$
V_T (L)			
Rest	0.91 ± 0.21	0.92 ± 0.27	0.73 ± 0.22
Steady state exercise	2.4 ± 0.29	2.6 ± 0.32	2.4 ± 0.40
Peak Exercise	2.7 ± 0.16	2.7 ± 0.23	2.6 ± 0.22
f_b (breaths/min)			
Rest	14.8 ± 1.5	14.4 ± 1.2	17.1 ± 3.0
Steady state exercise	33.0 ± 2.7	33.5 ± 2.5	31.2 ± 1.9
Peak exercise	50.1 ±3.8	51.0 ± 3.4	48.8 ± 3.6
$V_{E\,BTPS}$ (L/min)			
Rest	13.5 ± 1.5	13.2 ± 1.3	12.5 ± 0.74
Steady state exercise	79.2 ± 5.3	87.1 ± 3.1	74.5 ± 4.7
Peak exercise	136 ± 11.4	137 ± 10.6	126 ± 10.3

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p>0.05$. V_T, tidal volume; f_b, respiratory frequency; $V_{E\,BTPS}$, minute ventilation.

Table IV-9. Oxygen uptake and carbon dioxide output changes occurring during exercise in Control subjects.

	LSD	NSD	$NaHCO_3$
VCO_{2STPD} (L/min)			
Rest	0.26 ± 0.03	0.25 ± 0.05	0.25 ± 0.01
Steady state exercise	2.60 ± 0.14	2.75 ± 0.11	2.45 ± 0.32
Peak Exercise	4.05 ± 0.30	2.87 ± 0.80	3.22 ± 0.70
VO_{2STPD} (L/min)			
Rest	0.30 ± 0.04	0.29 ± 0.05	0.28 ± 0.03
Steady state exercise	2.88 ± 0.15	2.96 ± 0.12	2.75 ± 0.23
Peak Exercise	3.91 ± 0.27	3.77 ± 0.36	3.53 ± 0.31
VO_{2STPD} (ml/min/kg)			
Rest	4.3 ± 0.3	4.2 ± 0.2	4.3 ± 0.2
Steady state exercise	41.7 ± 0.8	42.4 ± 0.6	42.0 ± 0.8
Peak exercise	54.6 ±1.4	54.1 ± 1.4	54.1 ± 1.3

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p>0.05$. VCO_2, carbon dioxide output; VO_{2STPD}, oxygen uptake.

Table IV-10. Physiologic dead space/tidal volume ratio, respiratory exchange ratio and end-tidal carbon dioxide tension changes occurring during exercise in Control subjects.

	LSD	NSD	$NaHCO_3$
RER			
Rest	0.85 ± 0.03	0.84 ± 0.04	0.85 ± 0.04
Steady state exercise	0.89 ± 0.05	0.93 ± 0.04	0.88 ± 0.04
Peak exercise	1.03 ± 0.05	1.03 ± 0.05	1.00 ± 0.03
V_E/VO_2			
Rest	40.7 ± 3.9	40.0 ± 3.1	37.8 ± 3.0
Steady state exercise	26.8 ± 1.0	28.5 ± 1.3	26.3 ± 1.8
Peak Exercise	35.4 ± 2.3	37.3 ± 2.0	33.6 ± 3.0
V_E/VCO_2			
Rest	46.2 ± 2.9	47.5 ± 2.8	44.4 ± 2.2
Steady state exercise	30.2 ± 1.0	30.5 ± 1.0	30.3 ± 1.1
Peak exercise	34.2 ±2.3	36.4 ± 2.0	33.8 ± 2.5

Values are mean ± SEM. There were no significant differences in Control subjects for ventilatory and metabolic values by diet, $p>0.05$. RER, respiratory exchange ratio (VCO_2/VO_2); V_E/VO_2 and V_E/VCO_2, ventilatory equivalents for oxygen and carbon dioxide respectively.

Table IV-11. Arterial oxygen saturation changes occurring during exercise in Control subjects.

	LSD	NSD	$NaHCO_3$
S_aO_2 (%)			
Rest	92.6 ± 0.9	91.0 ± 1.3	88.0 ± 2.1
Steady state exercise	91.1 ± 2.2	95.0 ± 1.7	90.6 ± 1.9
Peak Exercise	92.0 ± 1.7	95.0 ± 1.9	91.5 ± 1.4

Values are mean ± SEM. There are no significant differences in Control subjects for S_aO_2 value by diet and exercise intensity, $p>0.05$. S_aO_2, arterial blood oxygen saturation.

Table IV-12. Cardiovascular changes occurring during exercise in EIA subjects.

	LSD	NSD	$NaHCO_3$
HR (bpm)			
Rest	92 ± 9.0	91 ± 4.8	94 ± 6.5
Steady state exercise	157 ± 1.9	163 ± 1.9	157 ± 3.1
Peak Exercise	172 ± 5.6	175 ± 6.5	172 ± 7.0
HR % predicted max (bpm)			
Rest	49 ± 3.7	49 ± 2.2	51 ± 3.7
Steady state exercise	86 ± 1.0	87 ± 1.0	86 ± 1.0
Peak exercise	91 ± 3.3	99 ± 1.9	93 ± 4.4
O_2 Pulse (ml/beat)			
Rest	3.4 ± 0.6	3.4 ± 0.5	3.2 ± 0.6
Steady state exercise	13.5 ± 2.2*	13.0 ± 2.3*	13.6 ± 1.7*
Peak Exercise	16.2 ± 2.8*	16.4 ± 1.9*	16.2 ± 2.5*

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. There were no significant differences in ventilatory and metabolic values by diet, $p>0.05$. HR, heart rate; HR%, HR as a percentage of maximum heart rate (220-Age); O_2 Pulse, oxygen pulse (VO_2/HR).

Table IV-13. Breathing pattern and exercise ventilation occurring during exercise in EIA subjects.

	LSD	NSD	$NaHCO_3$
V_T (L)			
Rest	0.63* ± 0.26	0.60 ± 0.36*	0.63 ± 0.22
Steady state exercise	2.0 ± 0.30*	2.1 ± 0.32*	2.0 ± 0.27*
Peak Exercise	2.3 ± 0.30*	2.3 ± 0.29*	2.3 ± 0.30*
f_b (breaths/min)			
Rest	17.3 ± 2.1	18.8 ± 2.4	17.3 ± 2.0
Steady state exercise	30.0 ± 1.9*	27.2 ± 1.6*	29.7 ± 2.1*
Peak exercise	41.1 ±3.3*	42.8 ± 3.4*	41.8 ± 3.3*
$V_{E\,BTPS}$ (L/min)			
Rest	10.9 ± 0.36	11.2 ± 0.65	10.9 ± 0.35
Steady state exercise	60.1 ± 5.1*	57.1 ± 5.6*	59.4 ± 4.1*
Peak exercise	94.5 ± 5.6*	98.4 ± 6.6*	96.1 ± 7.3*

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. There were no significant differences in ventilatory and metabolic values by diet, $p>0.05$. V_T, tidal volume; f_b, respiratory frequency; $V_{E\,BTPS}$, minute ventilation.

Table IV-14. Oxygen uptake and carbon dioxide output occurring during exercise in EIA subjects.

	LSD	NSD	$NaHCO_3$
VCO_{2STPD} (L/min)			
Rest	0.26 ± 0.02	0.26 ± 0.03	0.25 ± 0.02
Steady state exercise	1.87 ± 0.31*	1.85 ± 0.34*	1.84 ± 0.21*
Peak Exercise	2.67 ± 0.41*	2.85 ± 0.41*	2.71 ± 0.42*
VO_{2STPD} (L/min)			
Rest	0.31 ± 0.04	0.31 ± 0.03	0.30 ± 0.03
Steady state exercise	2.12 ± 0.34*	2.12 ± 0.35*	2.14 ± 0.27*
Peak Exercise	2.78 ± 0.39*	2.87 ± 0.35*	2.79 ± 0.40*
VO_{2STPD} (ml/min/kg)			
Rest	4.4 ± 0.3	4.5 ± 0.2	4.2 ± 0.3
Steady state exercise	34.2 ± 1.5*	33.1 ± 1.6*	32.8 ± 0.9*
Peak exercise	42.3 ±1.5*	42.8 ± 1.4*	41.6 ± 1.7*

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. There were no significant differences in ventilatory and metabolic values by diet, $p>0.05$. VCO_2, carbon dioxide output; VO_{2STPD}, oxygen uptake.

Table IV-16. Arterial oxygen saturation changes occurring during exercise in EIA subjects.

	LSD	NSD	$NaHCO_3^-$
S_aO_2 (%)			
Rest	96.0 ± 0.6	96.1 ± 0.6*	96.0 ± 0.6*
Steady state exercise	95.1 ± 0.9	94.8 ± 0.8	93.2 ± 1.7
Peak Exercise	95.8 ± 0.5	95.2 ± 1.5	94.7 ± 0.8

Values are mean ± SEM. *$p<0.05$ compared to respective value in Control subjects. There are no significant differences in S_aO_2 value by diet and exercise intensity ($p>0.05$). S_aO_2, arterial blood oxygen saturation.

Analysis of Variance for 2 x 2 Crossover Design: FEV1 @ 10 min post-exercise. EIA subjects					
Source	*d. f.*	*Sum of Squares*	*Mean Square*	*F value*	*P value*
Between-subjects:					
Carry-over	1	0.121	0.1210	0.0198	0.890
B.S. residual	6	6.103	1.0172		
Within-subjects:					
Direct Treatments (adjusted for Periods)	1	0.121	0.1210	1.775061	0.206
Periods (adjusted for Treatments)	1	0.035	0.0350	0.513447	0.486
W-S residual	6	0.409	0.0682		
		6.789			
Total	15	7.518			

Table IV-17. Analysis of variance for a 2 x 2 crossover design to assess carry-over effects for FEV_1 at 10 minutes post-exercise in EIA subjects.

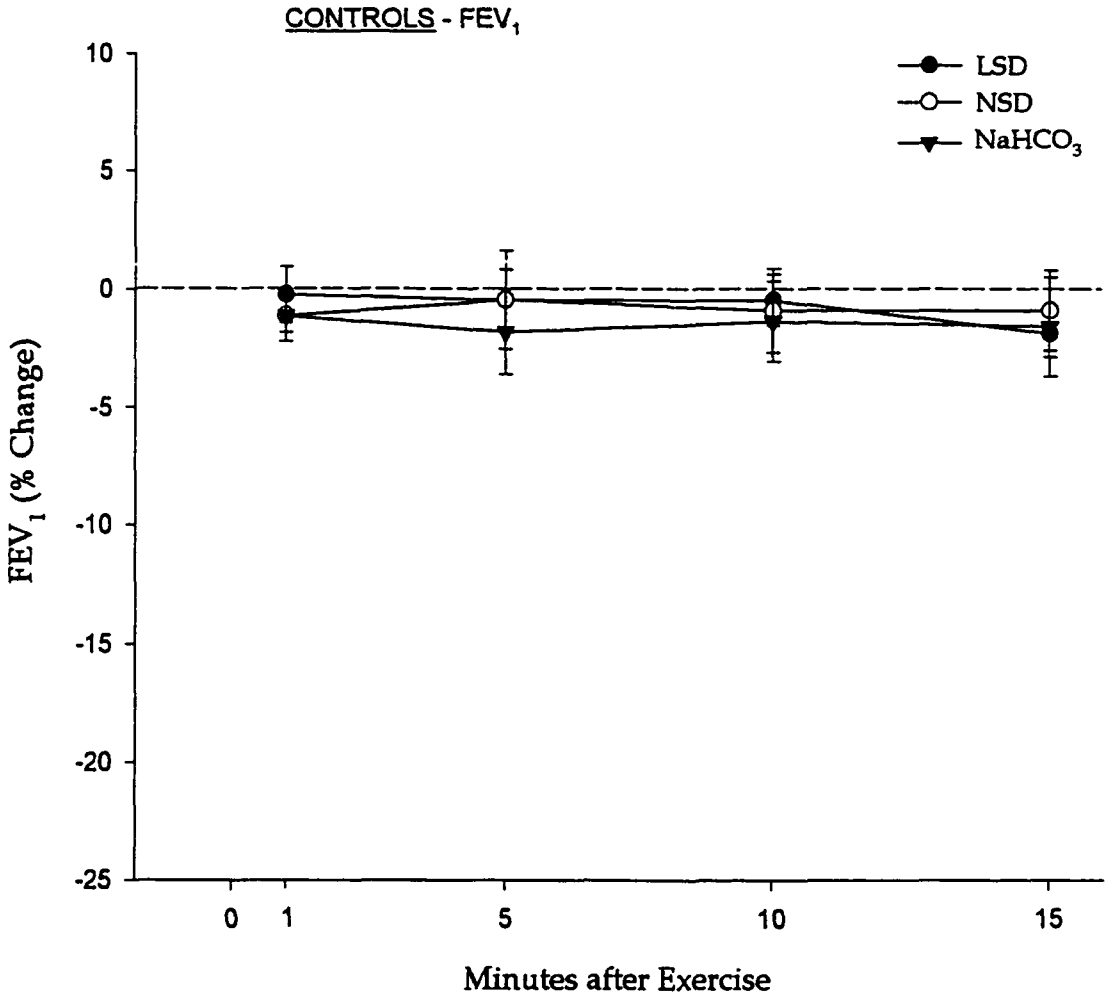


Figure IV-1. Mean percentage change in FEV_1 pre- to post-exercise in Control subjects.

Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$).

LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$ = sodium bicarbonate diet.

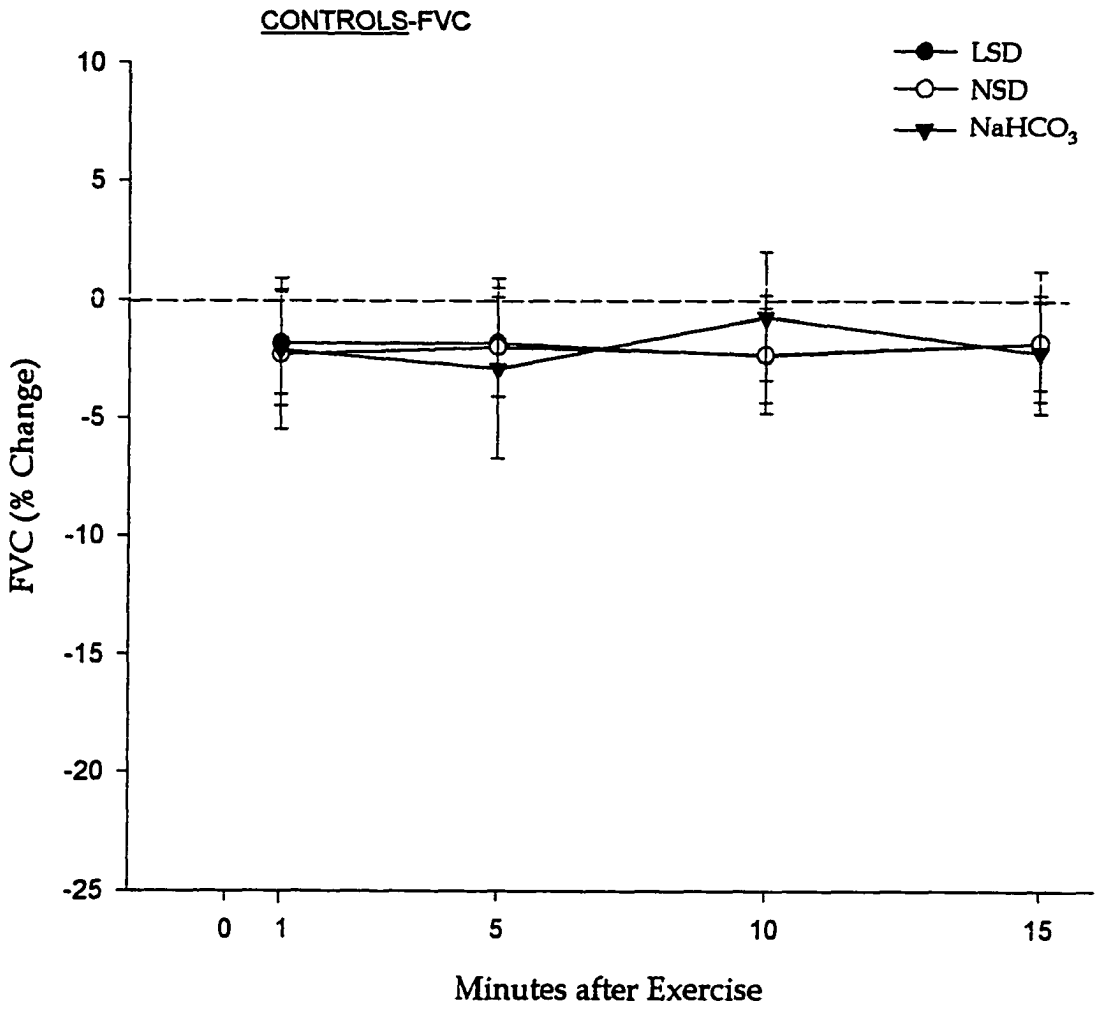


Figure IV-2. Mean percentage change in FVC pre- to post-exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

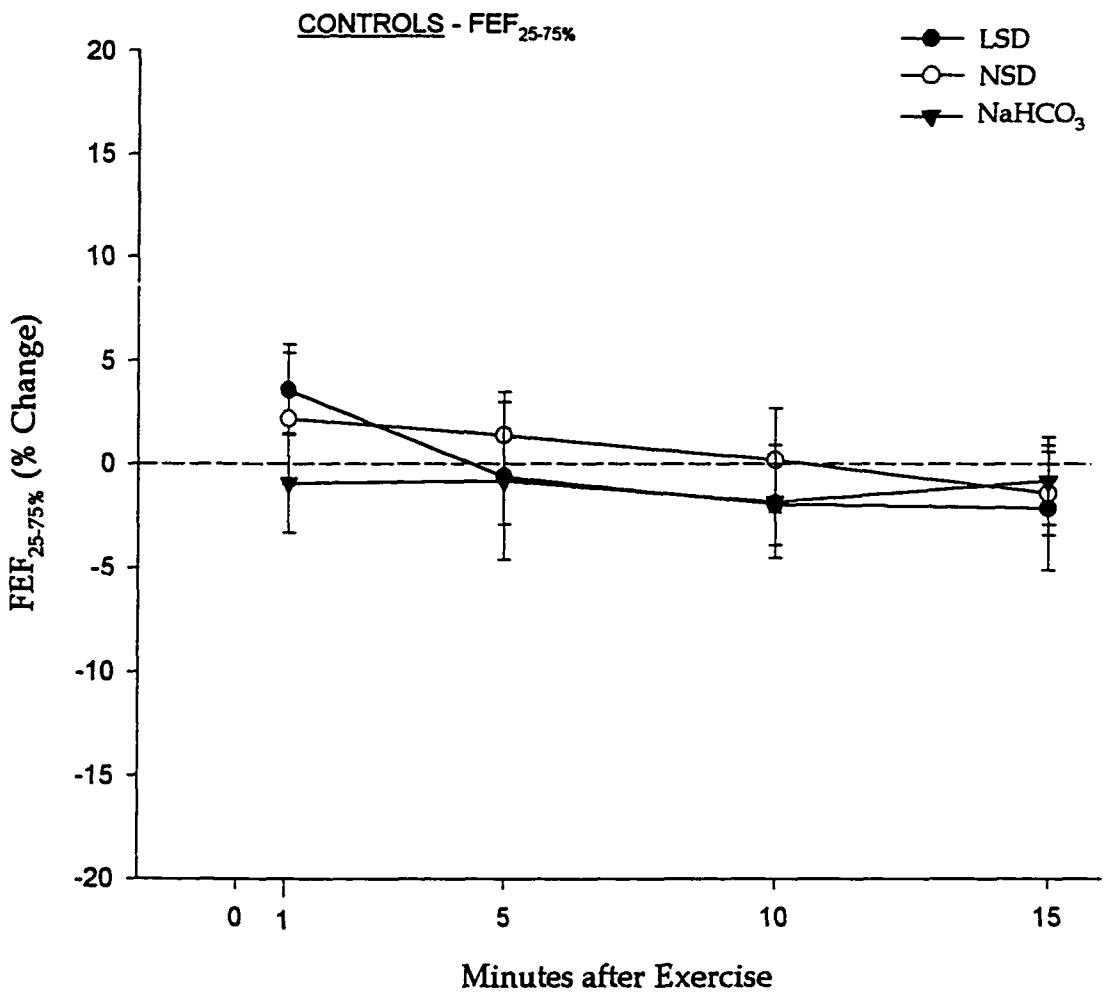


Figure IV-3. Mean percentage change in $FEF_{25-75\%}$ pre- to post-exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

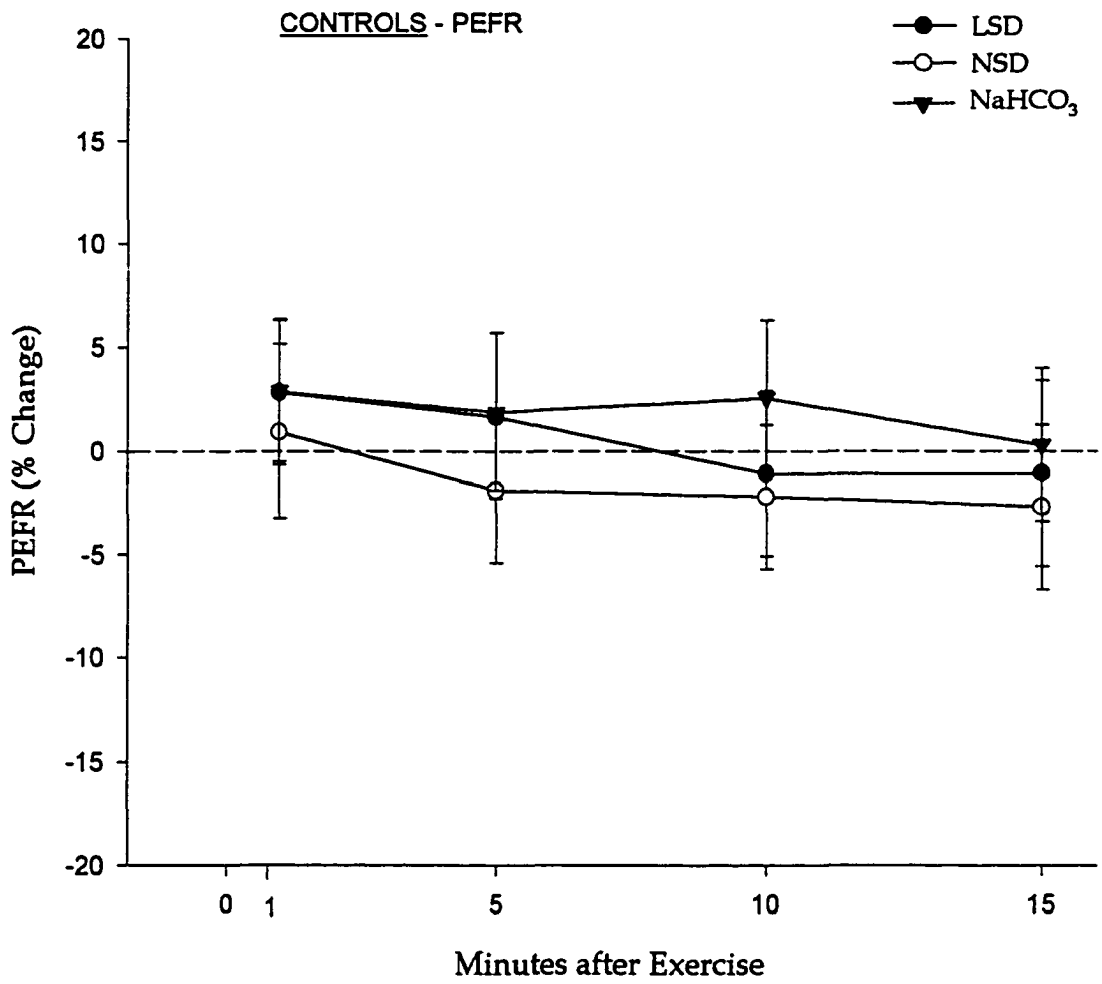


Figure IV-4. Mean percentage change in PEFR pre- to post-exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-lows salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

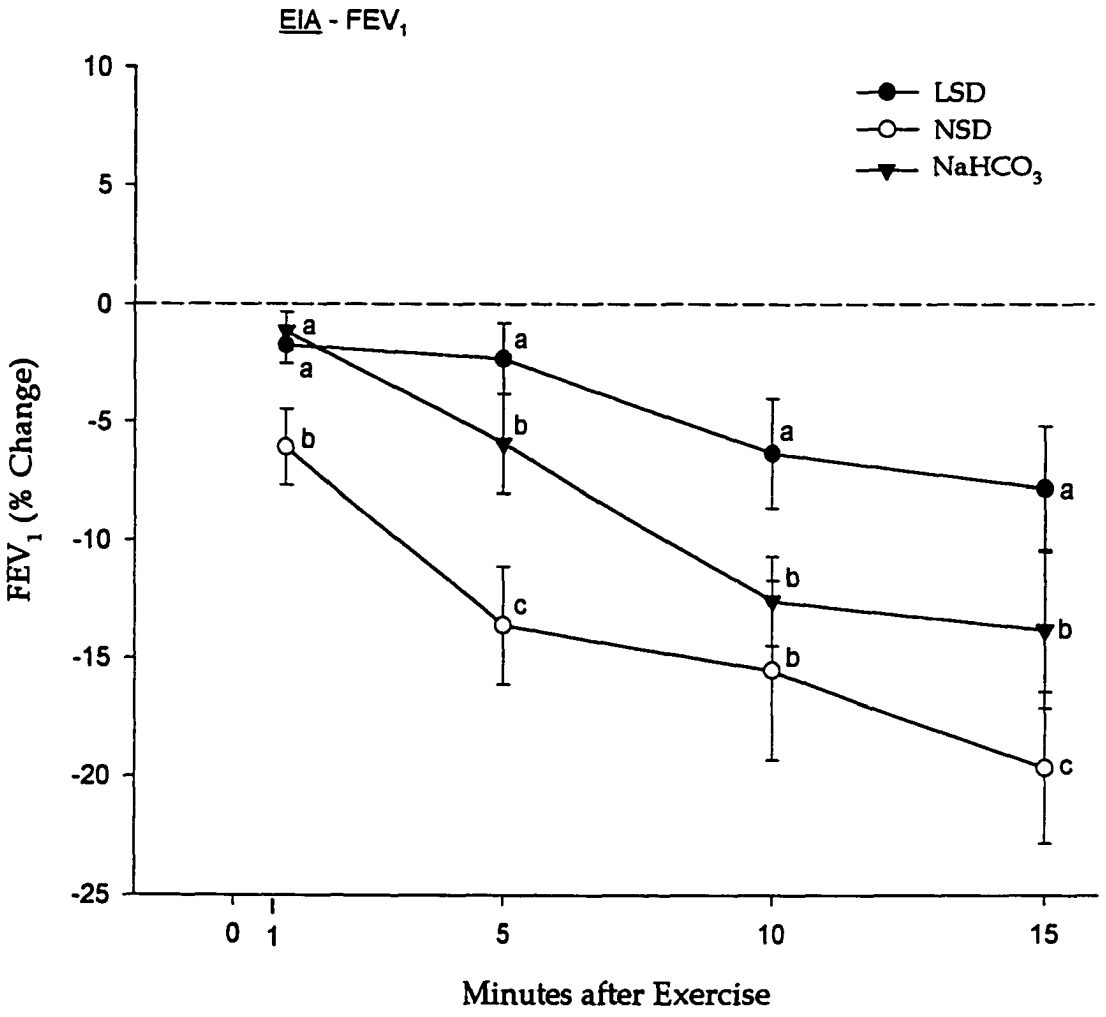


Figure IV-5. Mean percentage change in FEV_1 pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$), differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

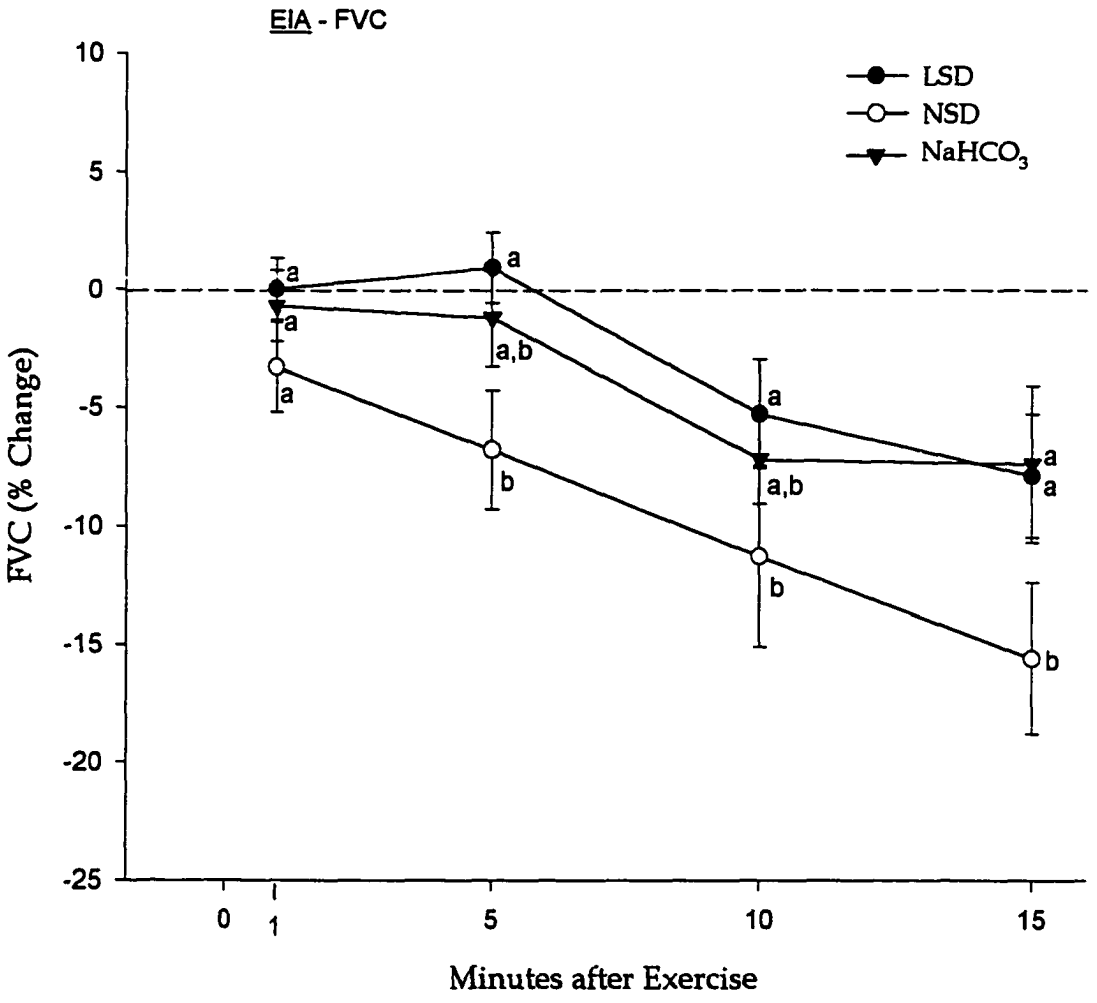


Figure IV-6. Mean percentage change in FVC pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

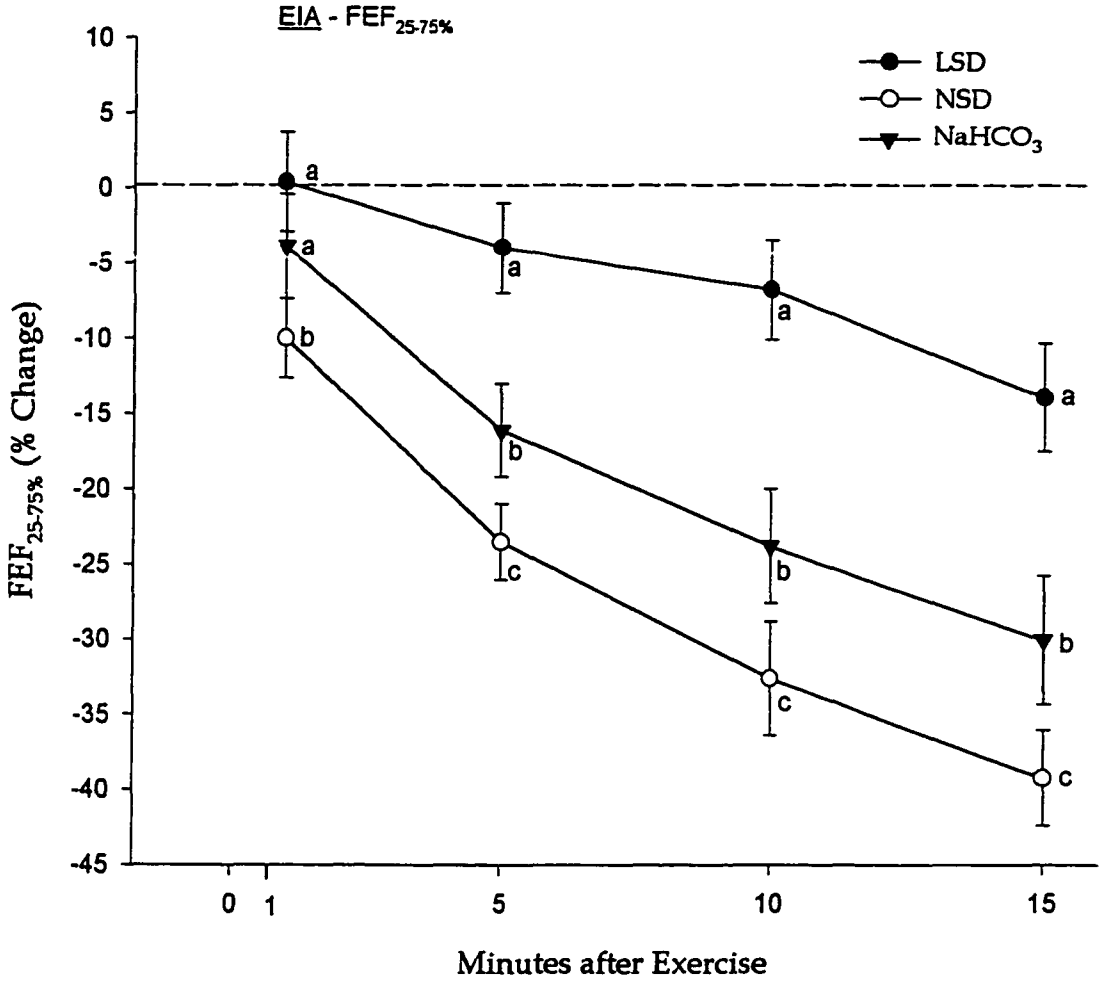


Figure IV-7. Mean percentage change in $FEF_{25-75\%}$ pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

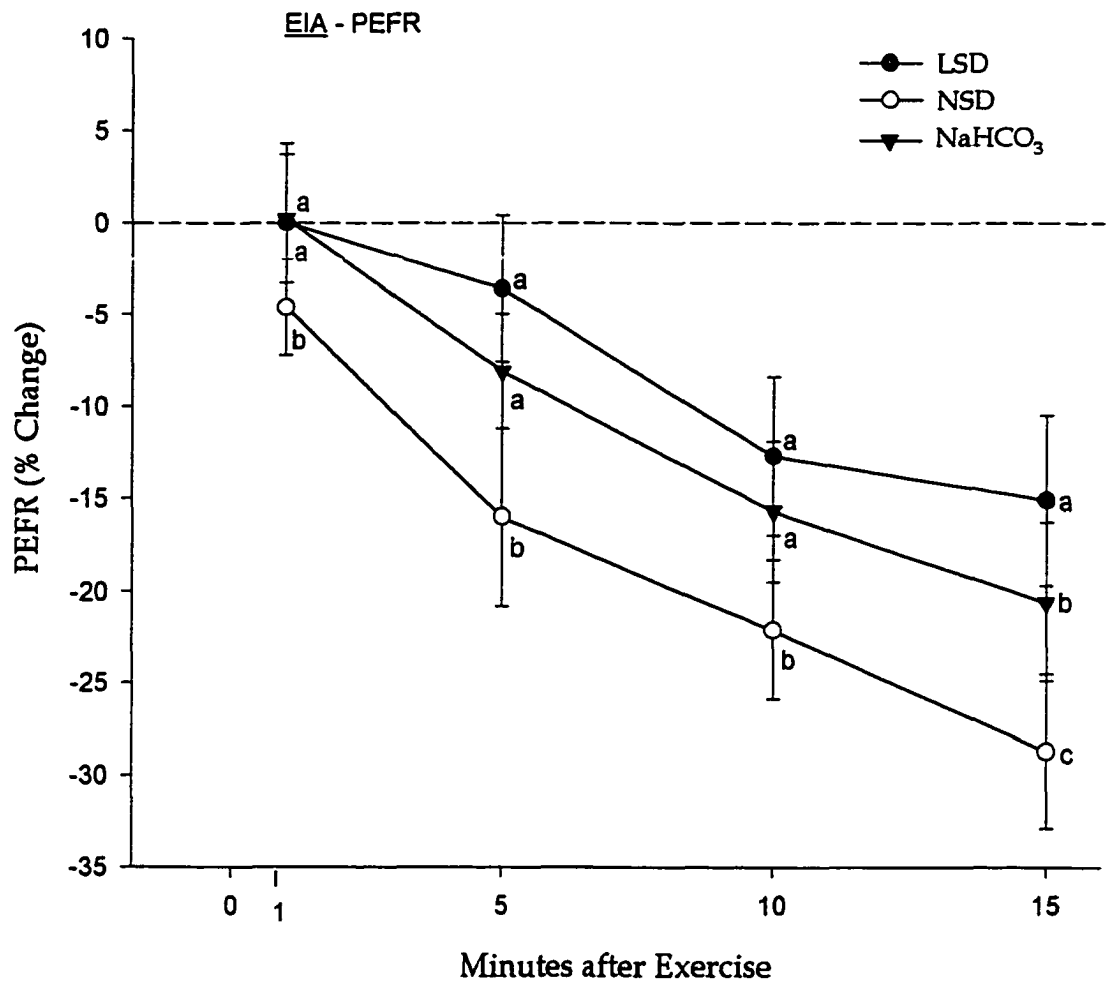


Figure IV-8. Mean percentage change in PEFR pre- to post-exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

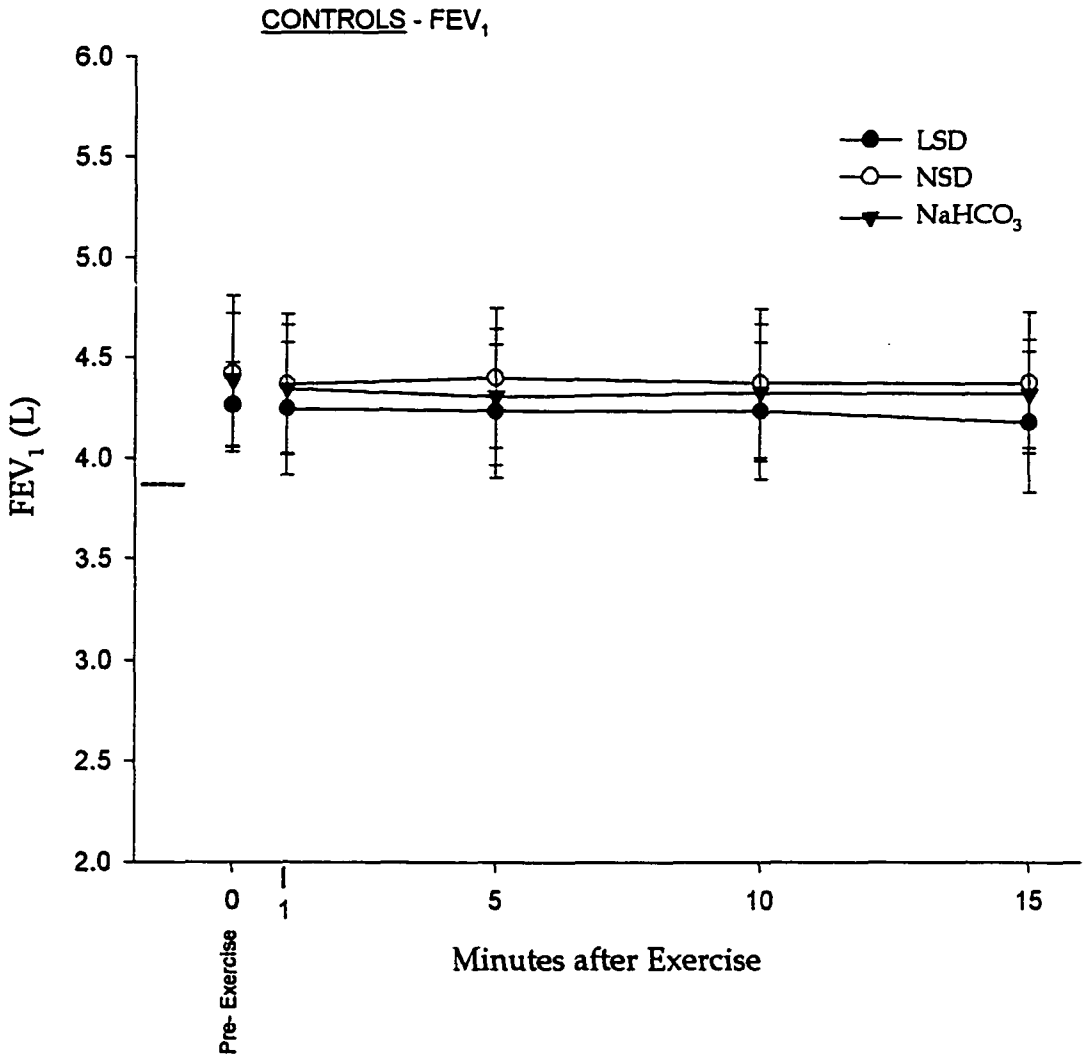


Figure IV-9. Changes in FEV_1 at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

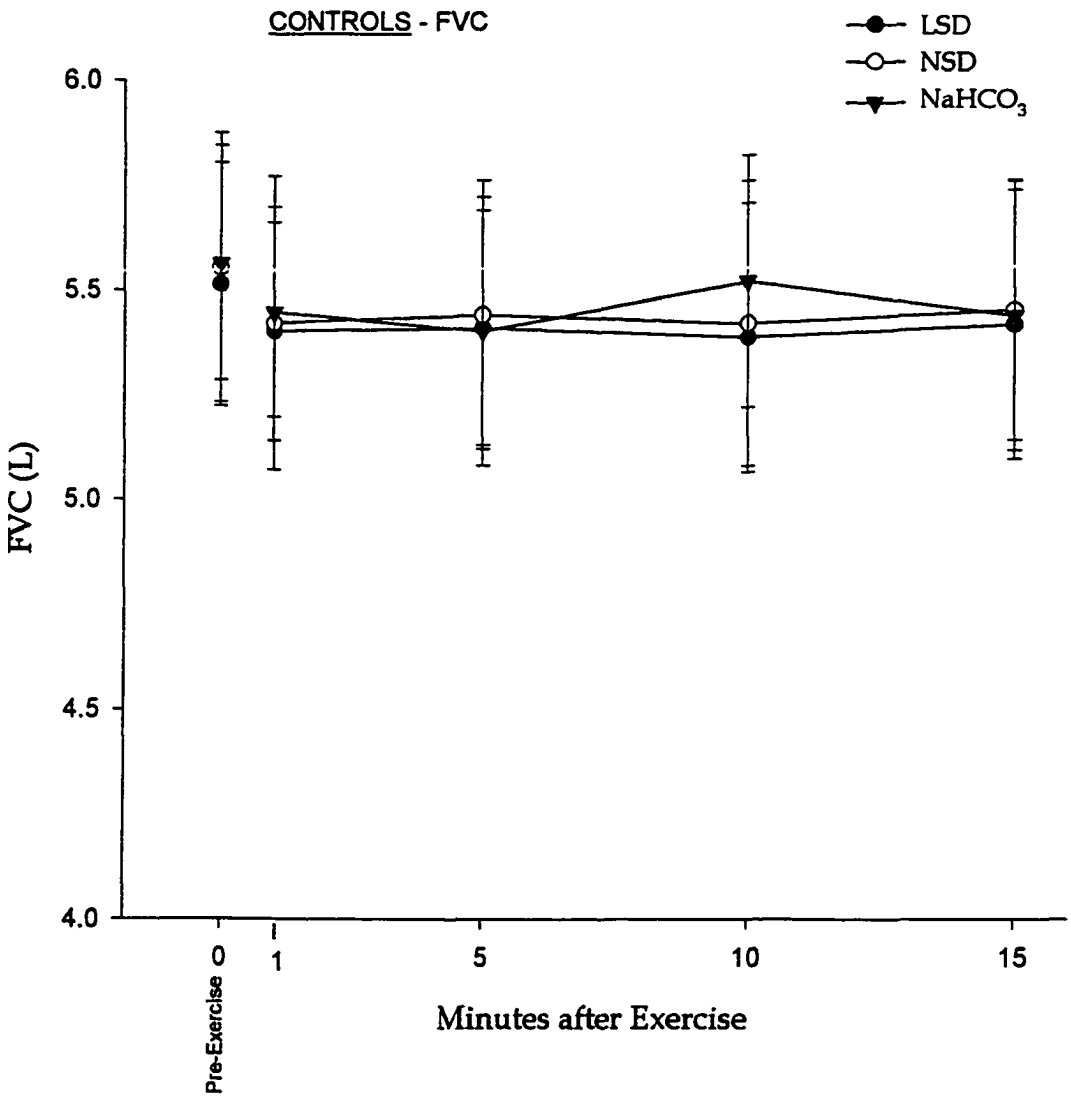


Figure IV-10. Changes in FVC at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

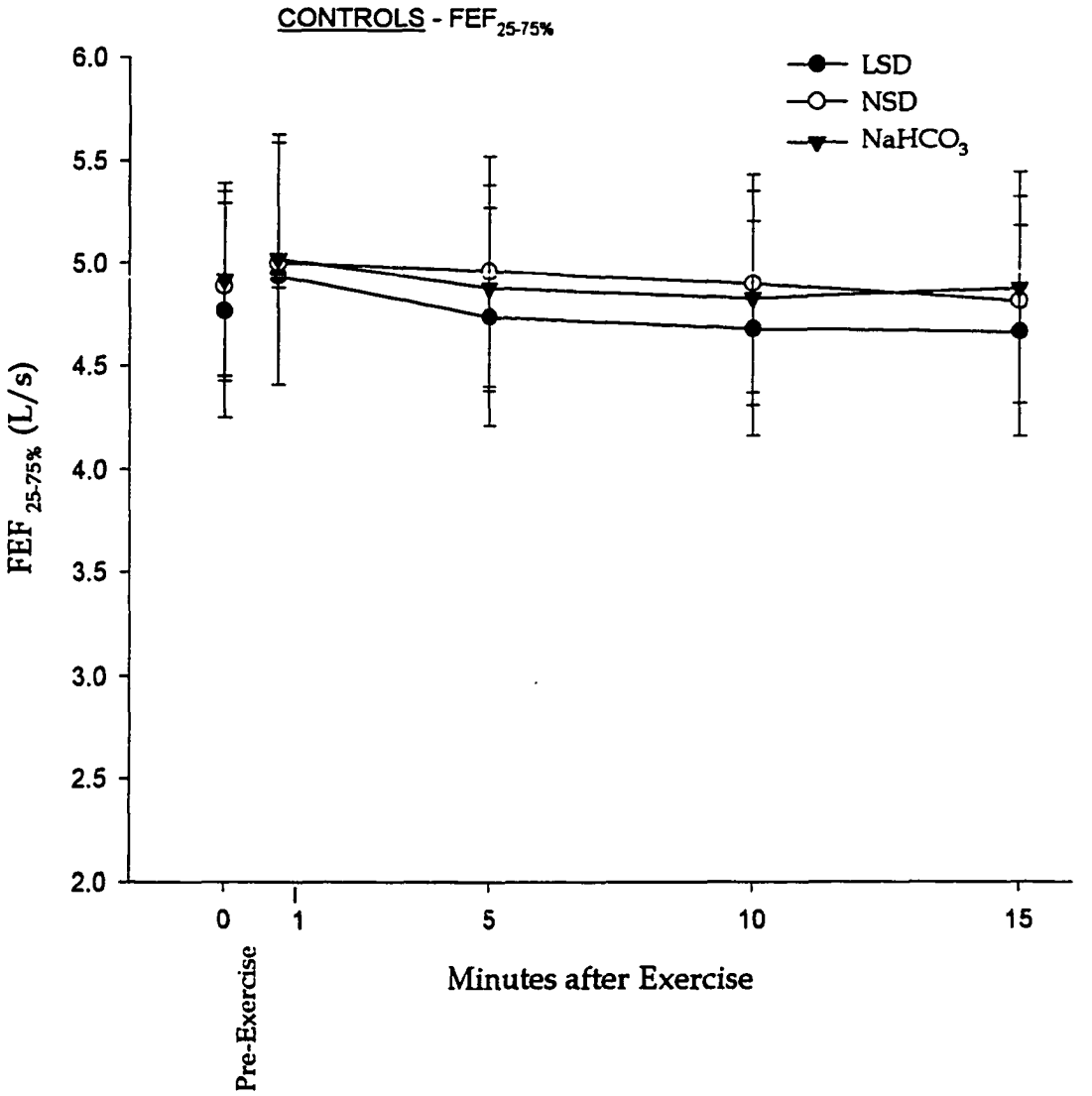


Figure IV-11. Changes in $FEF_{25-75\%}$ at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

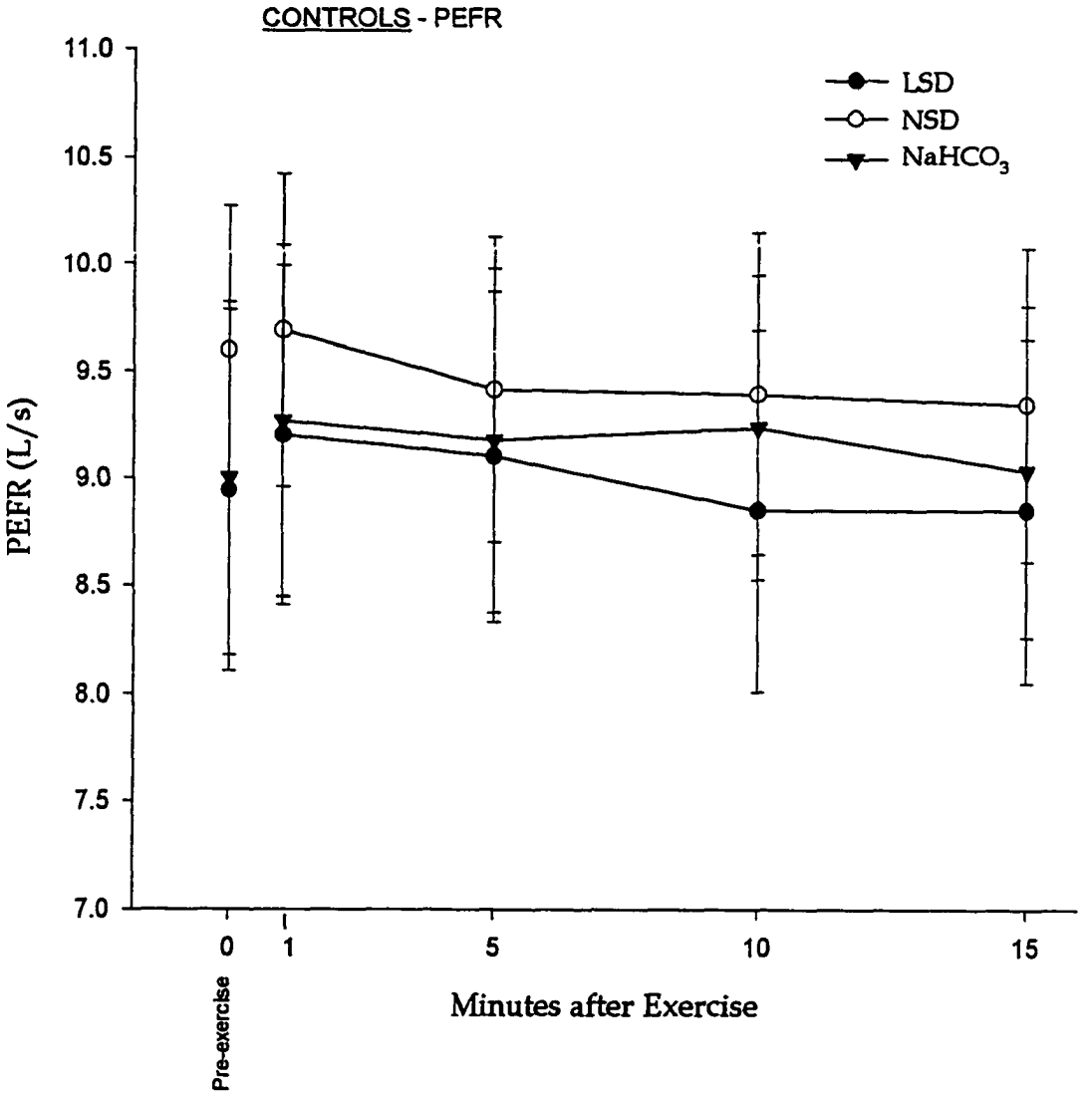


Figure IV-12. Changes in PEFR at pre-exercise and following exercise in Control subjects. Values are mean ± SEM. There were no significant differences by time or diet ($p>0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

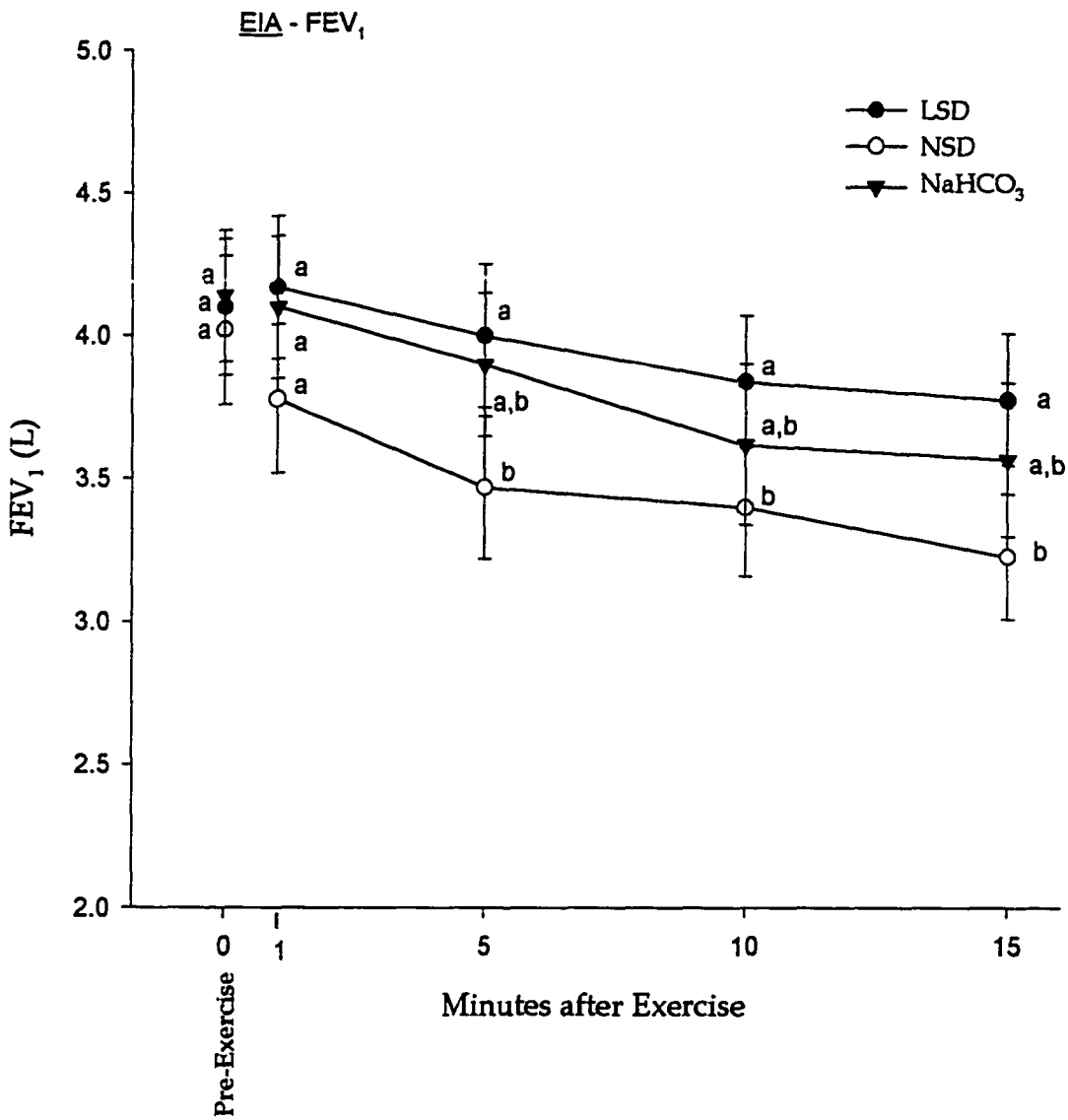


Figure IV-13. Changes in FEV_1 at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

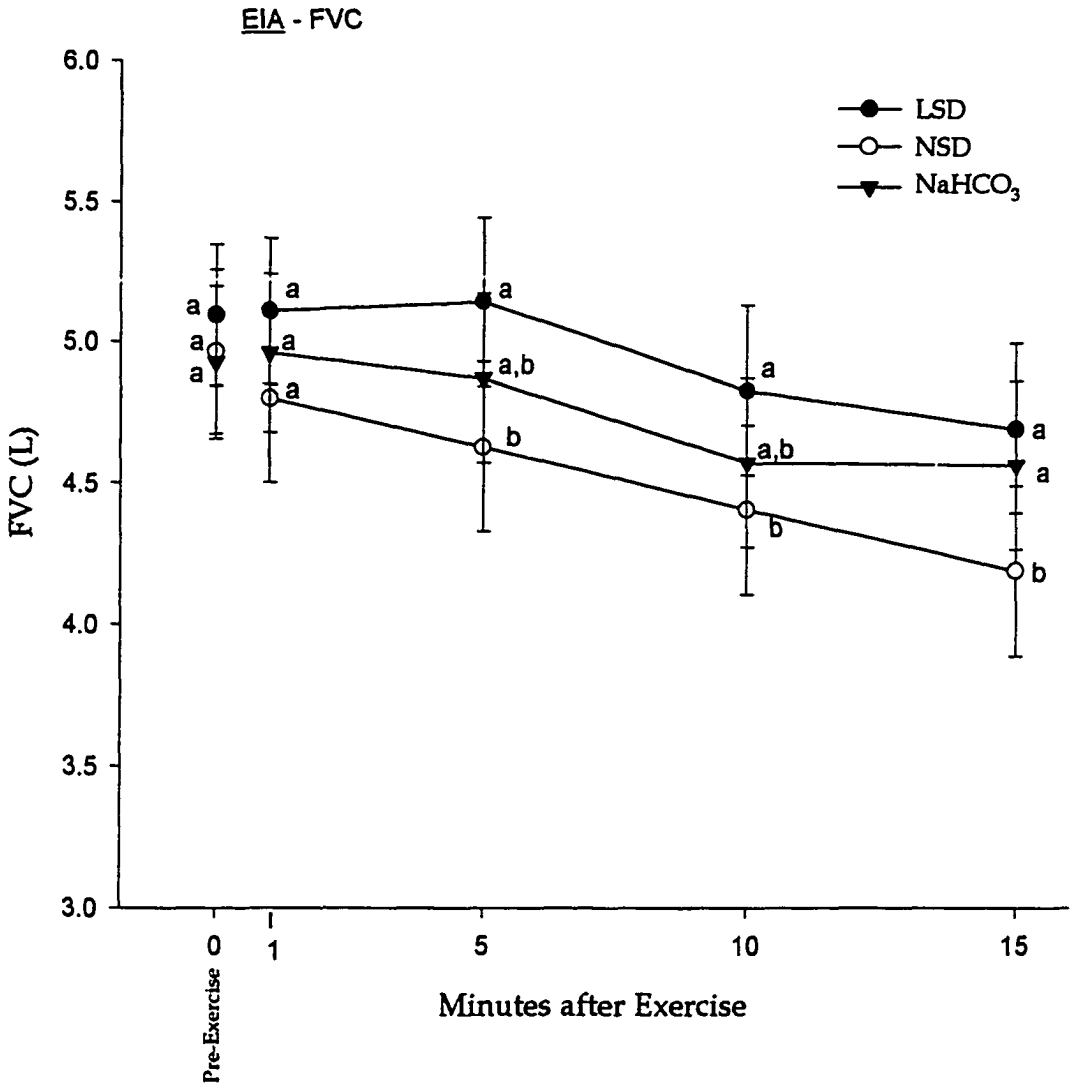


Figure IV-14. Changes in FVC at pre-exercise and following exercisein EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

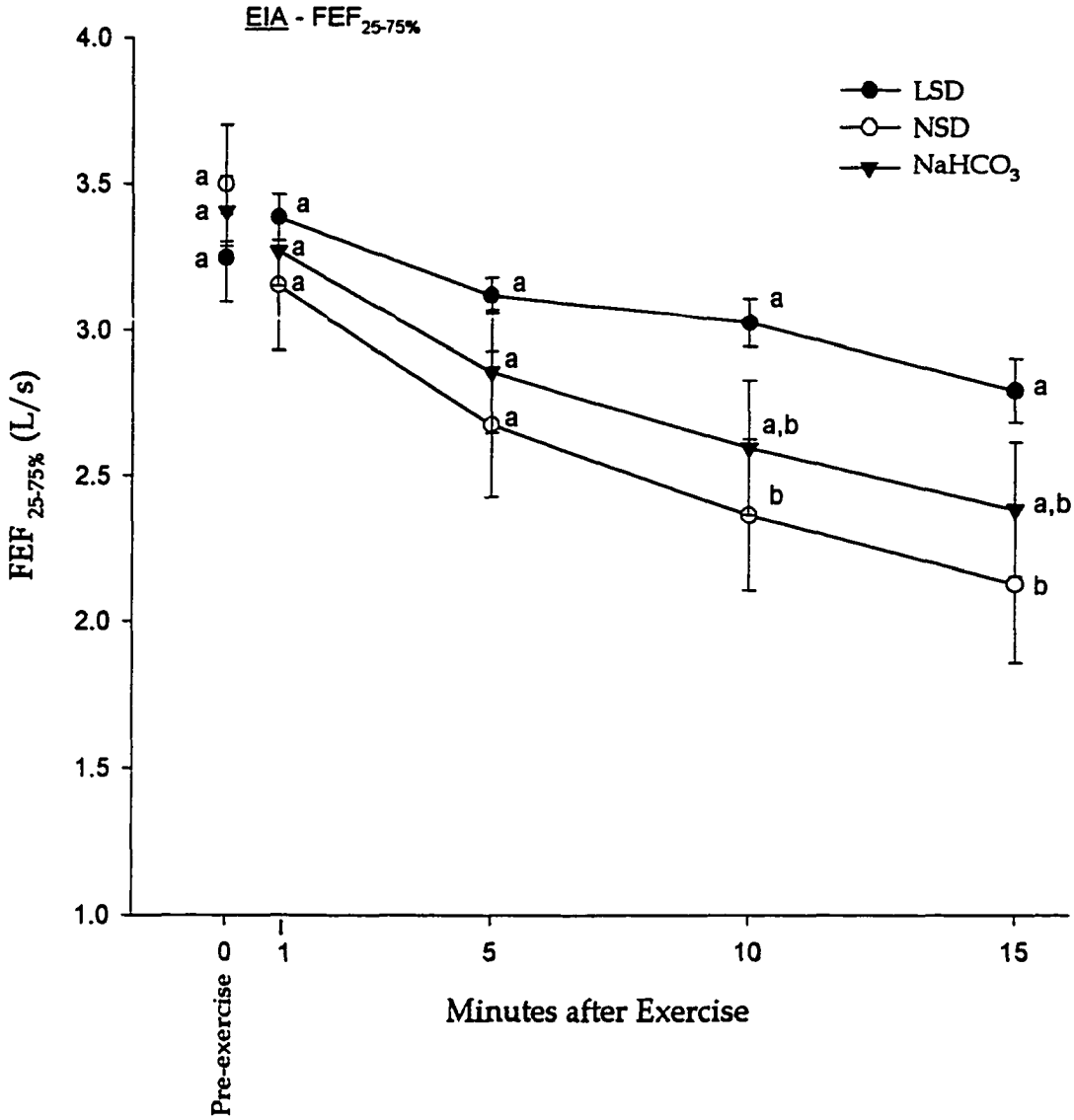


Figure IV-15. Changes in $FEF_{25-75\%}$ at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

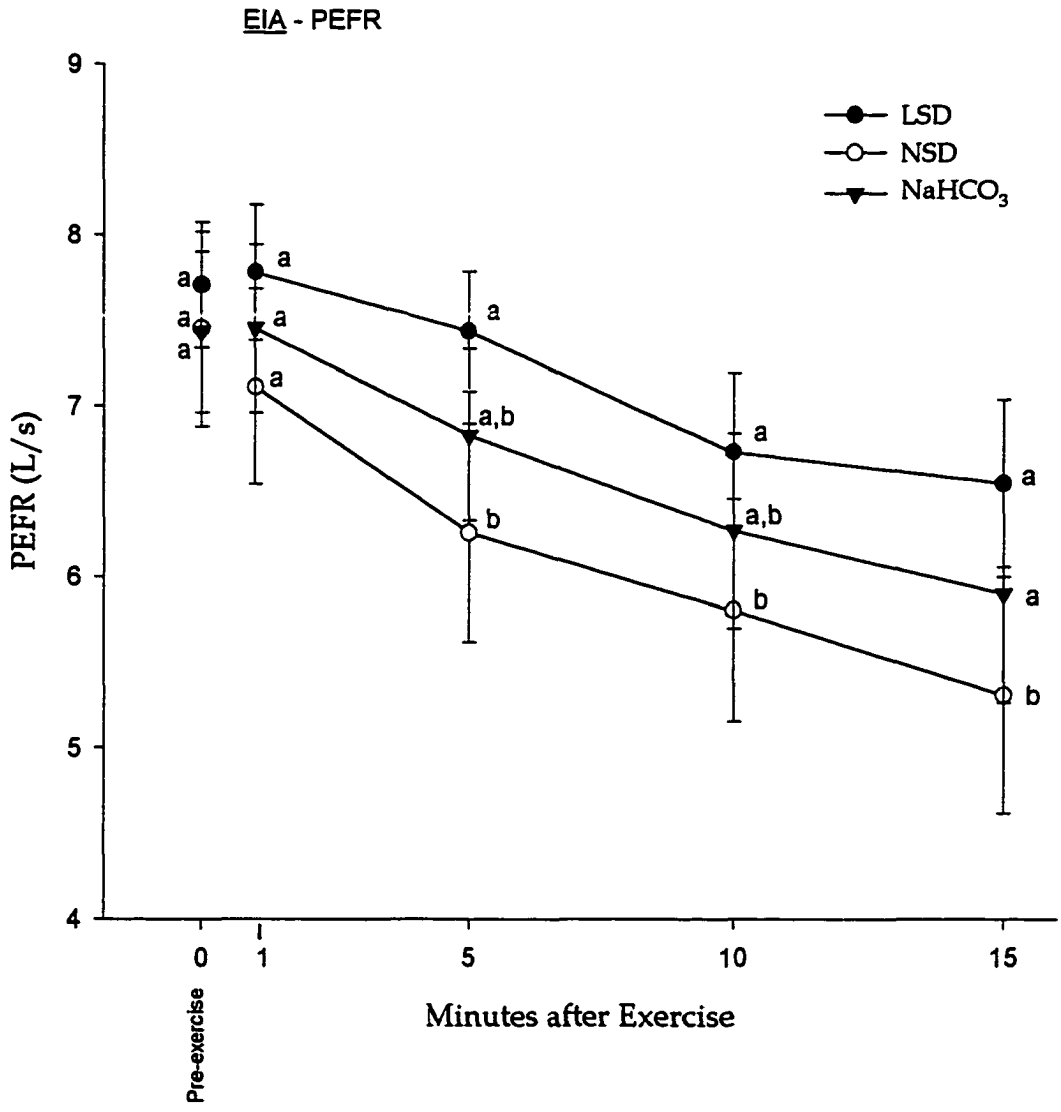


Figure 1V-16. Changes in PEFR at pre-exercise and following exercise in EIA subjects. Values are mean ± SEM. Letters denote significance for comparison of diets within time period. Values with the same letter are not significantly different ($p>0.05$); differing letters show significance among values ($p<0.05$). LSD-low salt diet; NSD-normal salt diet; $NaHCO_3$-sodium bicarbonate diet.

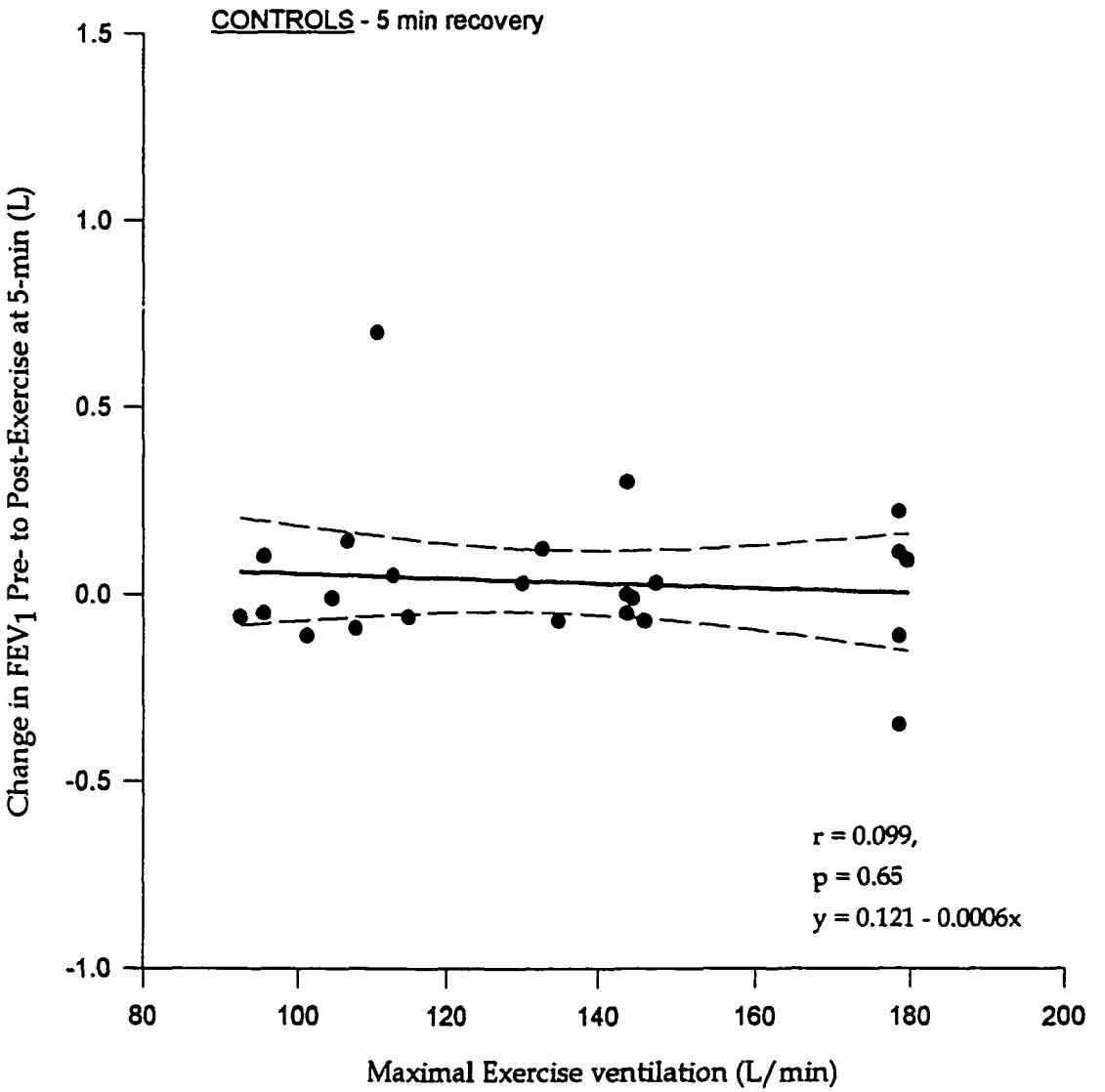


Figure IV-17. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 5-min of recovery in Control subjects.

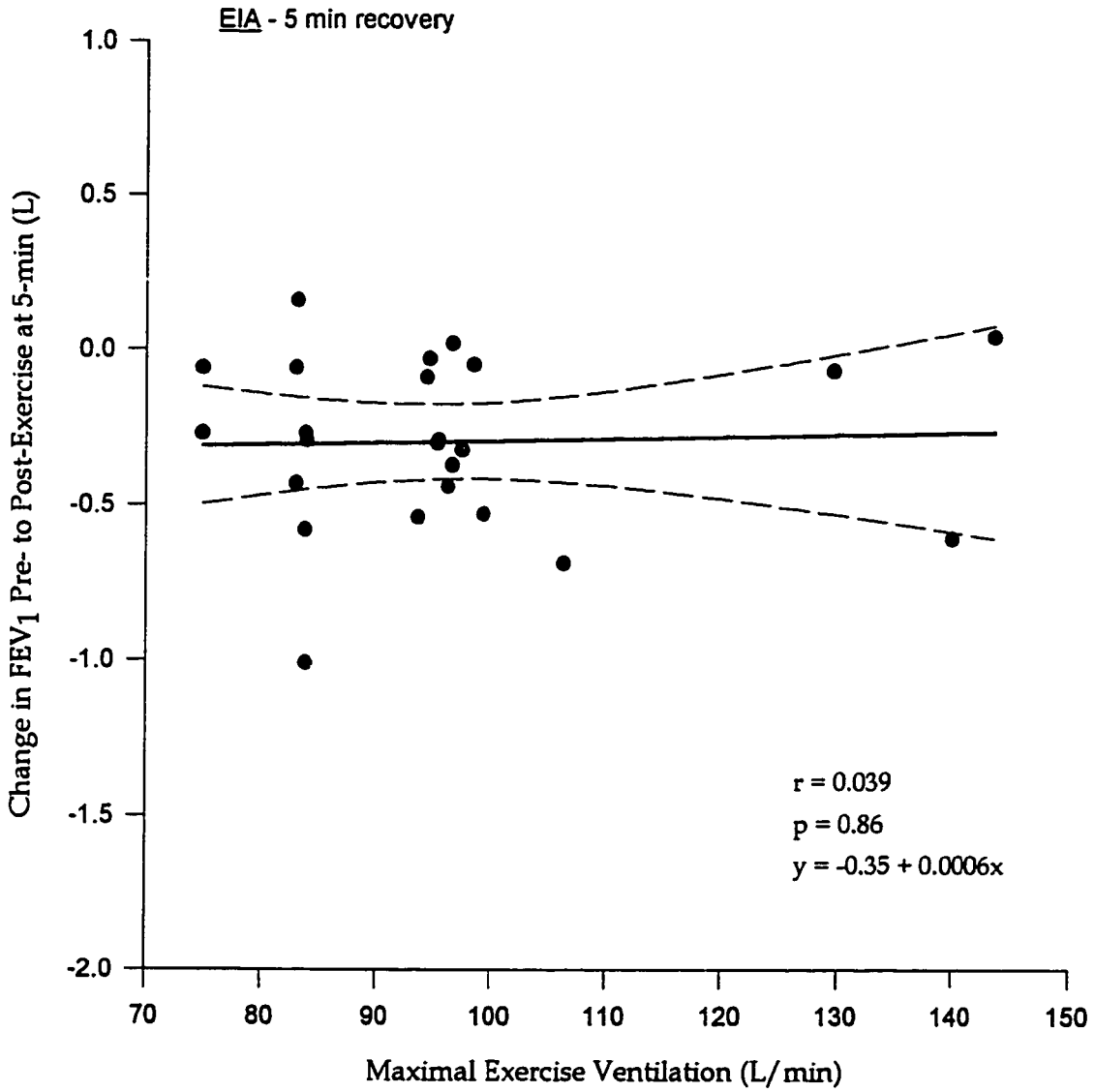
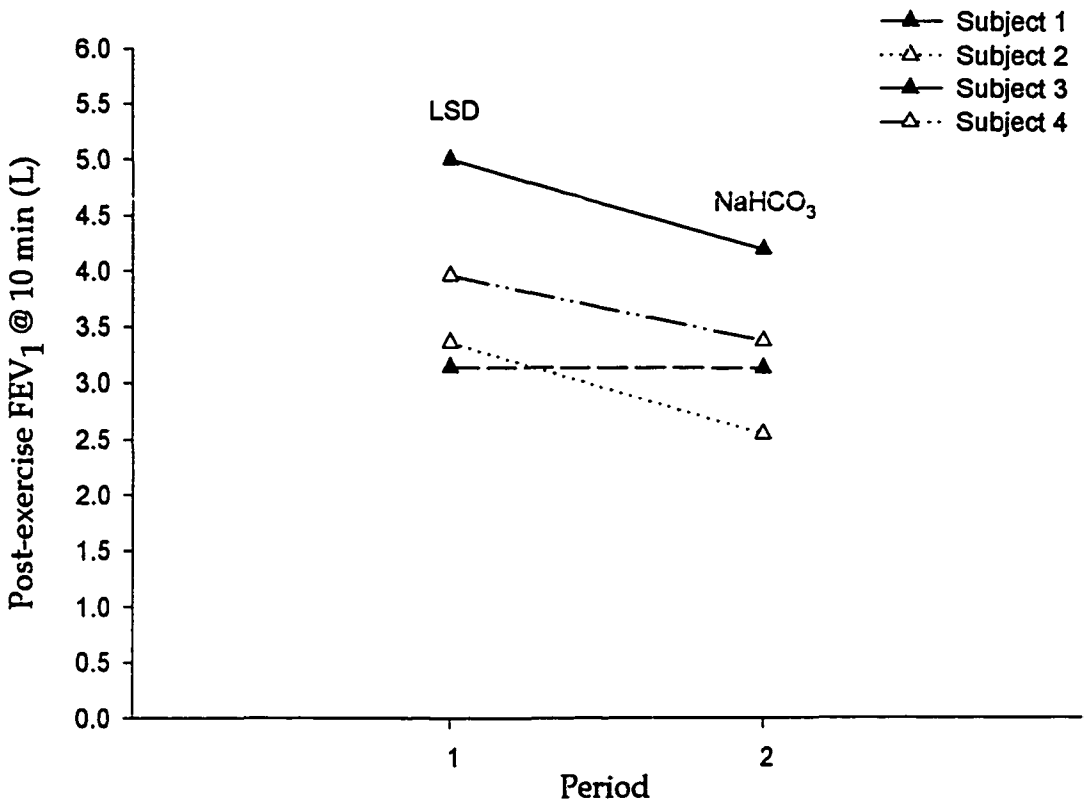


Figure IV-18. Regression of maximal exercise ventilation and pre-to post-exercise changes in FEV_1 at 5-min of recovery in EIA subjects.



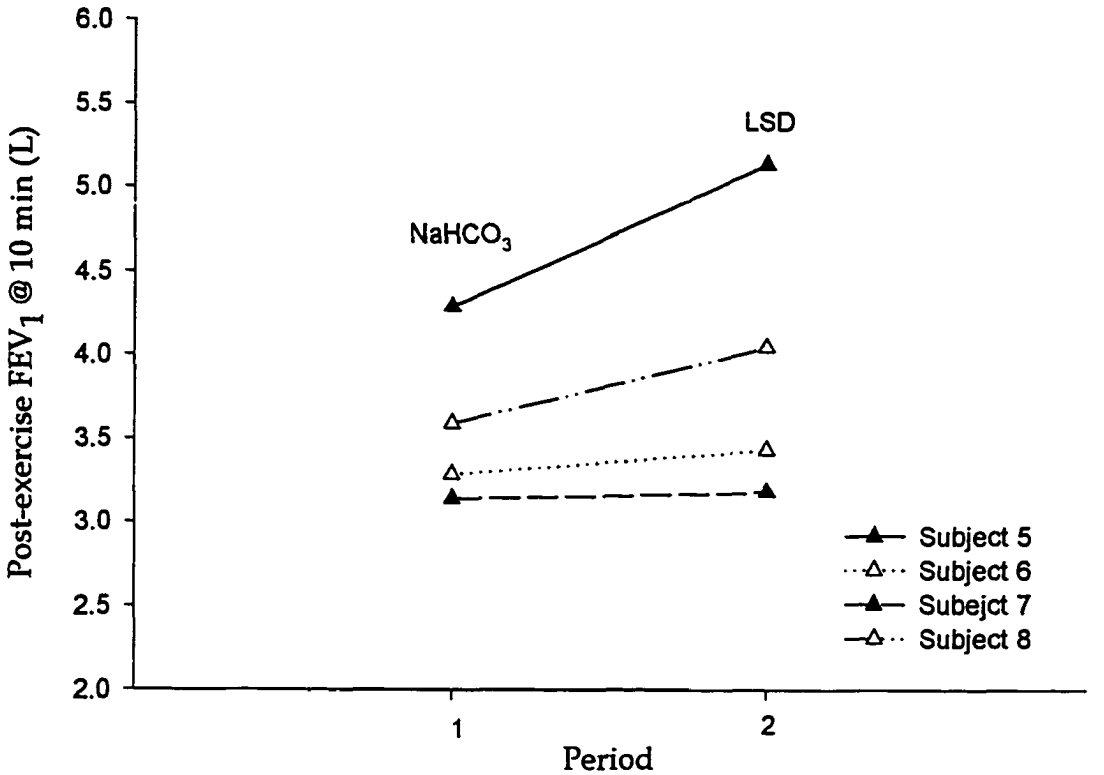


Figure IV-20. EIA Group Two subject profiles for FEV_1 at 10 minutes post-exercise.

Subjects started the $NaHCO_3^-$ diet in period 1 and then crossed over to the LSD in period 2.

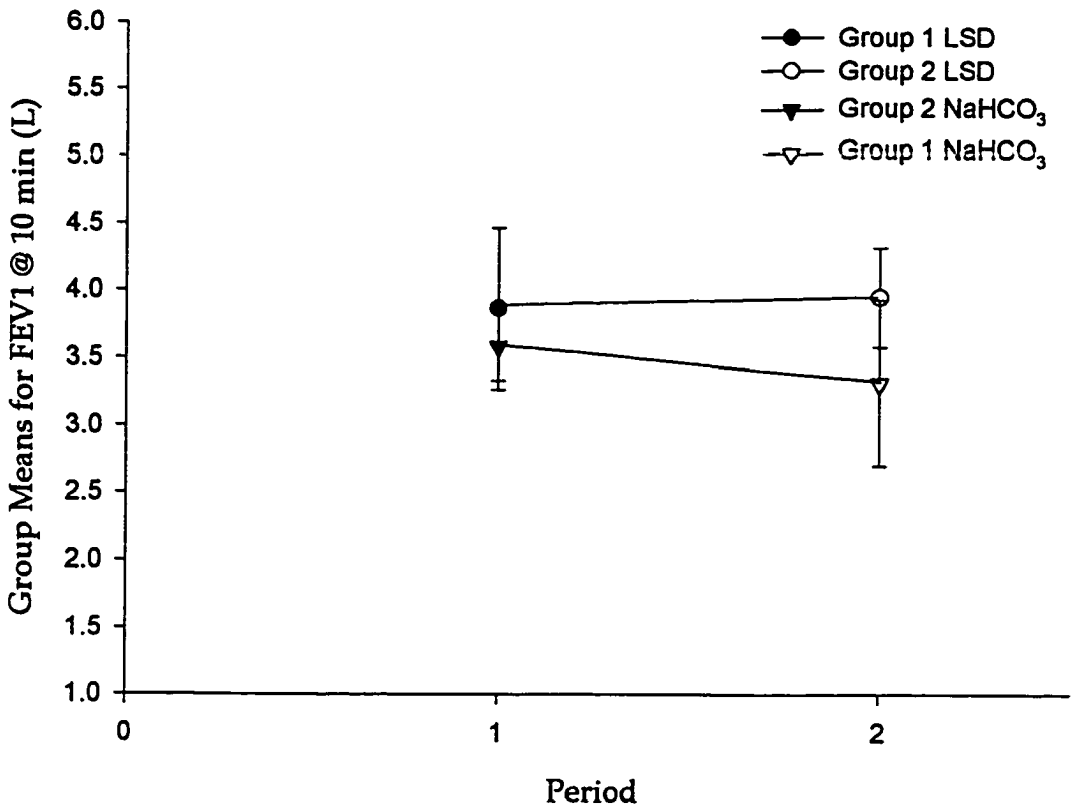


Figure IV-21. Group-by-period plot for EIA subjects at 10 minutes post-exercise FEV_1.

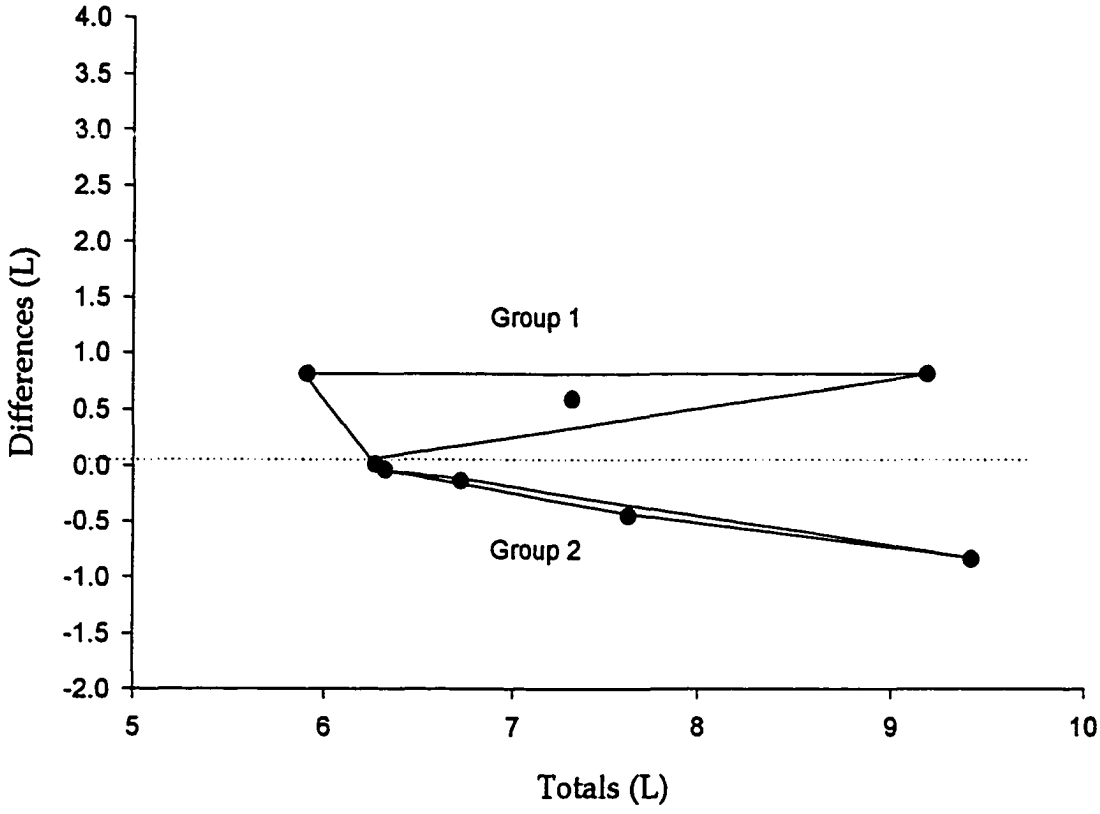


Figure IV-22. Differences (period 1 minus period 2) against totals (period 1 plus period 2) plot, in order to assess carry-over effects in EIA subjects at FEV_1 at 10 minutes post-exercise.

CHAPTER V

Dietary salt consumption and leukotriene-dependent hyperpnea-induced airway obstruction in guinea pigs

Abstract

Purpose: The purpose of this study was to determine if altering dietary salt (NaCl) consumption mediates leukotreine (LT) release in the airways of guinea pigs during hyperpnea-induced airway obstruction (HIAO). **Methods:** Thirty-two male Hartley strain guinea pigs (428-661 g) were split into two groups. One group (n=16) of animals ingested a normal salt (NS) diet (0.75% NaCl) for two weeks, while the other group of animals (n=16) ingested a high salt (HS) diet (2% NaCl) for two weeks. Thereafter, animals were anesthetized with sodium pentobarbital (45 mg/kg) and xylazine (7 ml/kg), cannulated (linguofacial vien or carotid artery), tracheotomized, and mechanically ventilated using a tidal volume (V_T) of 3 ml and a breathing frequency (f_b) of 60 breaths/min, during a 20 min baseline period. Ten minutes of dry gas hyperpnea (95% O_2/5% CO_2) ensued with a V_T of 4 ml and a f_b of 150 b/min. After the hyperpnea challenge, baseline ventilator settings were resumed using a gas mixture of 50% O_2 /50% Room Air (humidified and warmed) for 20 min. Ten minutes after the hyperpnea challenge, animals were administered either saline [NS- CON (n=8) and HS-CON (n=8)], or NDGA, a LT-LO inhibitor [NS-BLO (n=8) and HS-BLO (n=8)] for 10 min.

Subsequently, a second hyperpnea challenge for 10 min was followed by a second post-hyperpnea recovery period for 10 min. Bladder urine was collected by needle aspiration after euthanasia, and analyzed for urinary concentrations of sodium, chloride, potassium and creatinine. **Results:** There were no significant differences between the NS and HS diet for airway function prior to the first hyperpnea challenge (baseline), as determined by airway inspiratory pressure (Ptr) ($p>0.05$). Following the first hyperpnea challenge both groups demonstrated significant bronchoconstrictor responses compared to baseline. However, the HS diet elicited higher peak Ptr than the NS diet ($p<0.001$) at 10 min post-challenge. After infusion of saline (CON), and following the second hyperpnea challenge, both the NS-CON and HS-CON groups exhibited airway obstruction, as indicated by increased peak Ptr, compared to pre-hyperpnea values. However, the HS-CON group demonstrated higher peak Ptr than the NS diet group ($p<0.001$). Following infusion of the LT-inhibitor, and after the second hyperpnea challenge, HIAO was attenuated in both the NS-BLO and HS-BLO groups compared to the CON groups respectively ($p<0.001$). However, the HS-BLO group continued to demonstrate higher peak Ptr than the NS-BLO group ($p<0.05$). **Conclusion:** This study has demonstrated that dietary NaCl loading exacerbates the development of HIAO in guinea pigs, and that LT release is involved in HIAO and may be moderated by changes in dietary NaCl loading.

[***This study was supported in part by a grant from the College Research Council, College of Veterinary Medicine and Biomedical Sciences, Colorado State University***]

Introduction

Hyperpnea-induced airway obstruction (HIAO) in guinea pigs has been proposed as a potential animal model of exercise-induced asthma (EIA) in humans (13, 58), by using responses to dry gas isocapnic hyperventilation as a surrogate for exercise challenge. This model shares several common features with EIA, including the time course of onset of bronchoconstriction, the spontaneity of resolution, the diminution of response with humidification of inspired gas, the reproducibility of response with consecutive challenges, and the relationship between the amount of hyperpnea and the degree of response elicited (31, 58).

Anesthetized mammals in which both lungs are either exposed to or hyperventilated with cold dry air are commonly used as models to study HIAO. Hyperventilation with dry air evokes a complex airway response associated with microvascular leak and bronchoconstriction in several mammalian species, including guinea pigs, dogs, rabbits, and monkeys (7, 13, 28, 31, 48, 50). All models are mechanically ventilated via an endotracheal tube, so dry air bypasses some portion of the upper airway and is delivered directly into the lower trachea or the proximal airways. Although HIAO is artificially induced in all anesthetized animal models, their pulmonary responses to transient changes in ventilation are remarkably similar to that observed in normally exercising or hyperventilating asthmatic humans (13, 24, 25, 48, 58). In guinea pigs (56), HIAO does not usually develop until after hyperpnea stops. In man, it can occur during exercise (6), but it is more common when exercise stops (8).

When either asthmatic human or guinea pig airways are hyperventilated (26, 44) and allowed to recover breathing room temperature air, HIAO develops 2 to 6 min after

the challenge ends. Surprisingly, HIAO is markedly reduced in both species when airway cooling continues during the recovery period. This observation is consistent with the hypothesis that both airway cooling and subsequent rewarming are necessary for the initiation of HIAO (43). It is apparent that an increase in airway resistance in the guinea pig, as well as in subjects with exercise-induced asthma, may be caused by factors other than bronchial smooth muscle contraction, such as mucosal edema, mucus and fluid accumulation in the airways, and vascular congestion (15).

Bronchoactive eicosanoid mediators are synthesized by several cell types in the airways, and accumulating evidence suggests the potential participation of such eicosanoid mediators in hyperpnea-induced airway responses (30). First, when administered exogenously, the mediators may cause bronchovascuiar leak and bronchoconstriction (or bronchodilation) (37, 70). Second, it has been shown that after dry gas hyperpnea, guinea pig lavage fluid contains increased leukotriene and prostaglandin concentrations (33). Third, it appears that eicosanoid mediators can themselves elicit (9, 40) or modulate tachykinin release (22).

In addition to tachykinins, leukotrienes (LTs) have been found to act as mediators of HIAO in guinea pigs. Chapman and Danko (13) found that the LT receptor antagonist, FPL 55712, suppressed guinea pig HIAO. In addition, Garland et al. (30) showed that both BW-755c (antagonist for both cyclooxygenase and lipoxyygenase) and the LTD_4 receptor antagonist, ICI-198,615, suppressed HIAO. Similarly, Yang et al. (70) found increased LT levels immediately after 5 min of isocapnic hyperpnea. It is not clear, however, whether LTs act in parallel or in combination with tachykinins to induce airway narrowing in the process of HIAO.

Cells resident in the airways, such as mast cells, eosinophils, and alveolar macrophages, all have the requisite metabolic machinery to synthesize LTs. Human mast cells are extremely sensitive to changes in osmolarity, with small changes causing the release of mediators (21), thus establishing a possible link between the changes in osmolarity that may occur secondary to hyperventilation during exercise and subsequent mediator release. In addition, exercise seems to increase the propensity of mast cells, eosinophils, and alveolar macrophages to synthesize eicosanoid mediators (1).

The two previous studies (Chapters III and IV) have shown that increasing dietary salt (NaCl) consumption worsens, and NaCl restriction improves, post-exercise pulmonary function in EIA subjects. Therefore, this investigation was performed to determine if changes in dietary NaCl consumption in guinea pigs mediates the release of LTs from effector cells in HIAO. The experimental hypothesis tested in the present study was that a high salt (HS) diet would exacerbate HIAO compared to a normal salt (NS) diet, as exhibited by bronchoconstrictor responses to dry gas hyperpnea. It was further hypothesized that NaCl mediates the release of LTs from mediator cells in the airways. Nordihydroguaiaretic acid (NDGA), a non-selective inhibitor of LT biosynthesis and the lipoxygenase pathways, was used to suppress LT function.

Methods

Study Design and Manipulation of Salt Balance. Forty-two male Hartley strain guinea pigs (428g- 661 g) were purchased from Harlan (Harlan, Inc., Indianapolis, IN), of which 32 of the animals completed the study. All animals were maintained in

conventional laboratory animal facilities. All procedures in this study were approved by the Animal Care and Use Committee at Colorado State University and animals were treated according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Animals were housed in individual cages, permitting dietary manipulations on an individual basis.

The animals were divided into two groups. One group (n=16) of animals ingested a NS (0.75% NaCl) diet for two weeks - which is the normal NaCl content of guinea pig feed; while the other group of animals (n=16) ingested a HS (2% NaCl) (BIO-SERV, Frenchtown, NJ) diet. Both groups were fed 35 g/day of feed and supplied with water ad libitum. Each animal's food consumption and weight was monitored every 24 hours and fresh feed was replaced at the end of every 24-hour period.

To evaluate the role of a non-selective peptido-leukotriene (LT) biosynthesis inhibitor and lipoxygenase (LO) inhibitor, Nordihydroguaiaretic acid (NDGA) (Cayman Chemicals, Ann Arbor, MI), on HIAO in guinea pigs, the two NaCl diet groups (n=16) were further subdivided. The HS group was divided into two groups: One group (n=8) received NDGA (LT-LO blocked group; HS-BLO), while the other group (n=8) received saline (HS-CON) in a randomized fashion, and served as the Control group. The NS group was divided in an identical manner; NS-BLO (n=8) and NS-CON (n=8).

Animal Preparation. Animals were initially anesthetized with sodium pentobarbital (Veterinary Laboratories, Inc., Lenexa, KS; 65 mg/ml) given at 45 mg/kg intraperitoneally (IP) at 60% of the original dose (from a 13 ml/mg stock solution), followed 10-15 min later with xylazine (Vedco, Inc., St. Joseph, MO; 20 mg/ml) given at 7 mg/kg intramuscularly (IM) at 50% of the original dose , and supplemented with local

infiltration of the incision site with 0.5 ml of 2% lidocaine (Vedco, Inc., St. Joseph, MO; 100 mg/ml), prior to performing the surgical procedure. The anesthetic plane was maintained by boosting the animals at 5-10% of the original dose of sodium pentobarbital, as required. In order to assess the depth of anesthesia, heart rate, monitored non-invasively via an ECG (Spacelabs, Inc., Richmond, WA; Model No. 90603A), respiratory rate, toe pinch, movement during incision, and corneal reflex were used as a guide. The animals were placed on a heating pad (37°C) for the duration of the experiment. The linguofacial vein or carotid artery was cannulated with PE-50 polyethylene tubing to allow for the administration of drugs. A high cervical tracheostomy was performed, and the trachea was isolated and cannulated with a short piece of polyvinyl tubing [I.D. 1.67 mm (0.066"); O.D. 2.42mm (0.095")] attached to a 15-gauge cannula which was connected to a small animal ventilator (Harvard Rodent Ventilator, Model 683). The inspiratory and expiratory tubes of the ventilator were attached to the tracheal cannula through a Y-type connector with a 3 cm common segment (total dead space 0.3 ml) to minimize conditioning of inspired air. The inspiratory port of the ventilator was connected to a warmed (35-37°C) humidifier through which room air or a 50% O_2 /50% room air gas mixture passed. The tracheal inspiratory pressure (Ptr) was measured at the tracheal cannula with a pressure transducer (Statham P10 EZ, Gould Instrument Systems, Valley View, OH) and recorded on a Chart Recorder (Gilson ICT-2H Duograph, Middleton, WI) continuously, for the entire experimental procedure. Increases in Ptr, which reflect increases in respiratory system impedance, were interpreted to indicate airway narrowing, even though the possible contributions of microvascular leak and other mechanical changes were not

quantified. At the end of the protocol, the animals were euthanized with sodium pentobarbital (100 mg/kg, iv). After euthanasia, bladder urine samples (0.5-1 ml) were collected by needle aspiration from each animal and stored at -70°C until analysis. Urinary concentrations of electrolytes were measured by an autoanalyzer (Roche Diagnostics, Indianapolis, IN).

Experimental Protocol. Initial baseline ventilator parameters were set at 60 breaths/min (f_b) with a tidal volume (V_T) of 3 ml, and an inflation pressure of 10 cm H_2O at end-inspiration. During this run-in period, the animals breathed warmed and humidified Room Air. These ventilation conditions are referred to as "quiet breathing". The animals were allowed 20 min to stabilize during this baseline period. A fixed volume history of each animal (total lung inflation) was obtained twice on each animal, by occluding the expiratory port of the ventilator at the beginning of the run-in period.

Immediately thereafter, dry gas hyperpnea (hyperpnea challenge # 1) was mechanically imposed for a period of 10 minutes by using dry 95% O_2/5% CO_2 delivered at room temperature from a balloon reservoir into the inspiratory port of the mechanical ventilator. Inspiratory inflation pressure was increased to 15 cm H_2O and f_b was increased to 150 with a V_T of 4 ml for the challenge. After hyperpnea challenge # 1, the animals were returned to "quiet breathing" (post-hyperpnea period # 1) for 20 min using a humidified and warmed inspired gas mixture of 50% O_2/50% Room Air delivered from a balloon. During this period, airway narrowing was quantified as the highest airway Ptr recorded during the first 10 min post-hyperpnea period . Twenty min after the first hyperpnea challenge a second hyperpnea challenge (hyperpnea

challenge # 2) was performed in an identical fashion to the first challenge for a 10 min period, followed by 10 minutes of "quiet breathing" (post-hyperpnea period # 2).

To determine the effect of an LT-LO inhibitor, in HIAO, 1 ml of the solution (2 mg/kg of the NDGA crystalline solid dissolved in 0.2 ml of methanol and 0.8 ml of 0.9% saline) was administered to the HS-BLO (n=8) or NS-BLO (n=8) group of guinea pigs through either a linguofacial vein or carotid artery catheter 10 min after the first hyperpnea challenge and infused at a rate of 0.1 ml/min via a syringe pump (Cole-Parmer Instrument Company, Vernon Hills, IL, 74900 Series Multichannel Syringe Pump). The control groups, HS-CON (n=8) and NS -CON (n=8) received either 1 ml of 0.9% saline (n = 4) or 0.8 ml of 0.9% saline with 0.2 ml methanol added (n = 4).

Statistical analysis. Data were analyzed using the SYSTAT 8.0 statistical package (SPSS, Inc., Chicago, IL). During the initial baseline "quiet breathing" and post-hyperpnea period # 1 stage, each min of the last 5-min time period was averaged to give a single data point for airway inspiratory Ptr. Each min of the 10-min hyperpnea challenge #1 and #2 was averaged to give a single data point for airway Ptr. In addition, airway inspiratory Ptr was statistically analyzed every 2 min during post-hyperpnea periods #1 and #2. The effect of diet (NS and HS) and time (min) on airway inspiratory Ptr were analyzed by a two-way ANOVA with repeated measures across time (pre- and post-challenge) within each diet. The effect of diet, treatment (CON and BLO) and time were analyzed by a three-way ANOVA with repeated measures across time (pre- and post-hyperpnea challenge) within each diet. When a significant F-ratio was found ($p<0.05$), a Bonferroni post-hoc multiple pairwise comparison with paired t-tests was used to identify differences in group means ($p<0.05$). As there were no significant

differences ($p>0.05$) in inspiratory Ptr noted between the NS-CON and HS-CON groups for saline (n=4) and saline with methanol (n=4), the two sub-groups were pooled and analyzed as one sample population (HS-CON or NS-CON group). Unpaired t-tests were conducted between treatments on bladder urine electrolytes, food consumption, and body weight. Power was calculated at 0.902, using the following data: a minimum detectable difference in means = 0.02, expected standard deviation of residuals = 0.01, number of groups = 4, group size = 8, and p set at 0.05. All statistical tests of significance were set at $p<0.05$. Data are expressed as mean $\pm$ SEM.

Results

Body weights, food consumption, and bladder urine electrolyte concentrations are summarized in Table V-1. Initial and final body weights among the different treatments did not differ ($p>0.05$). Guinea pigs appear to have definite taste preferences, and alterations in the composition of a feed or the introduction of a new feed may result in a decline in food consumption. This may account for the fact that food consumption differed significantly between diets, with the NS diet group eating significantly more than the HS diet group ($p<0.001$). Bladder urinary sodium and chloride concentrations were significantly higher for the HS diet compared to the NS diet ($p<0.001$). No significant differences were noted for bladder urinary concentrations of potassium and creatinine ($p>0.05$).

Baseline conditions and airway response to hyperpnea challenge #1. The airway inspiratory Ptr values for baseline (V_T = 3 ml, f_b = 60 b/min) and hyperpnea challenge (V_T = 4 ml, f_b = 150 b/min), were not significantly different between the two

diet (NS and HS) groups ($p>0.05$). The time course of the change in Ptr during post-hyperpnea period #1 was consistent with the development of airway obstruction (Figure V-1). On both diets, the response was similar in that the inspiratory Ptr showed an increase as early as 2 min after the dry gas challenge, and peaked at 10 min, with a gradual return to baseline (pre-hyperpnea challenge #1) values by 20 min. The HS diet elicited significantly higher inspiratory Ptr than the NS diet during the post-hyperpnea period #1 during all time periods ($p<0.05$), indicating increased airway resistance.

Airway response to hyperpnea challenge # 2 after infusion of saline (CON groups). There was no significant difference between the HS and NS diet inspiratory Ptr value during hyperpnea challenge #2 ($p>0.05$). The time course of inspiratory Ptr for the CON groups are shown in Figures V-2 to V-4 and Figures V-6 and V-7. The HS-CON group exhibited higher inspiratory Ptr values than the NS-CON group at 8 min ($p<0.05$) and at 10 min during post-hyperpnea period #2 ($p<0.001$) (Figure V-4). In addition, both groups demonstrated significant increases ($p<0.001$) in inspiratory Ptr values at 6, 8, and 10 min compared to the Ptr value just prior to hyperpnea challenge # 2 for the respective diet, demonstrating airway obstruction.

Airway response to hyperpnea challenge # 2 after infusion of leukotriene inhibitor [NDGA] (BLO groups). Infusion of NDGA, a leukotriene biosynthesis and lipoxygenase inhibitor, blunted the bronchoconstrictor response seen in CON groups on both diets. However, the HS-BLO group demonstrated significantly higher inspiratory Ptr values than the NS-BLO group (Figure V-5) at 8 and 10 min ($p<0.05$). No significant difference ($p>0.05$) was noted at pre-hyperpnea challenge #2 between NS-BLO and HS-BLO. The NS-BLO group demonstrated significant reductions in inspiratory Ptr values

compared to the NS-CON group at 4 min ($p<0.05$) and 6, 8, 10 min ($p<0.001$) (Figure V-6). The HS-BLO group demonstrated significant reductions in inspiratory Ptr values at 6, 8, and 10 min compared to the HS-CON group ($p<0.001$) (Figure V-7).

Comparison of airway responses during post-hyperpnea period # 1 and # 2 for CON and BLO groups. Similar bronchoconstrictor responses were seen during post-hyperpnea period #1 and #2 for CON groups; there were no significant differences between inspiratory Ptr during the post-hyperpnea period #1 and #2 for NS-CON and HS-CON within respective time period up to 10 min (NS-CON, Figure V-2 and HS-CON, Figure V-3) ($p>0.05$). Both NS-BLO and HS-BLO groups demonstrated significant reductions in Ptr values in post-hyperpnea period #2 compared to post-hyperpnea period # , within respective time period from 4 min to 10 min (NS-BLO, Figure V-2 and HS-BLO, Figure V-3) ($p<0.001$).

Peak inspiratory Ptr values. During post-hyperpnea periods #1 and # 2, the greatest increase in inspiratory Ptr occurred at 10 min, so this time was chosen to test the statistical significance of the changes in peak inspiratory Ptr changes among the NS-CON and HS-CON groups. In addition, the same time period was used to signify peak inspiratory Ptr for the NS-BLO and HS-BLO groups. Figure V-8 depicts the peak inspiratory Ptr values for pre- and post-hyperpnea challenge #1 and #2. Significant differences ($p<0.001$) were noted between peak Ptr inspiratory values comparing baseline (average of the last 5 min) and post-hyperpnea challenge # 1 respectively for NS and HS diets. Significant differences ($p<0.001$) were observed for peak Ptr values between pre-hyperpnea challenge # 2 (average of the last 5 min) and post-hyperpnea challenge # 2 for NS-CON and HS-CON groups, but not for the NS-BLO and HS-BLO

groups ($p>0.05$). The peak inspiratory Ptr value during post-hyperpnea period # 2 was significantly higher ($p<0.001$) for the HS-CON group compared to the HS-BLO and NS-CON group. In addition, the NS-BLO group demonstrated a significant reduction in peak inspiratory Ptr, during post-hyperpnea period # 2, compared to the NS-CON group ($p<0.001$) and HS-BLO group ($p<0.05$). Figures V-9 and V-10 are examples of airway Ptr tracings for CON- and BLO groups on the NS diet.

Discussion

The results of the present study confirm that a dry gas hyperpnea challenge evokes an airway response in guinea pigs, and presents the novel finding that HIAO is enhanced by increased dietary NaCl. Furthermore, this study demonstrates that LT release from effector cells in the airways may be the end result of a final common pathway for normal HIAO and NaCl-enhanced HIAO. This suggests an important interaction between dietary salt consumption and LT release. The blockade of HIAO by a non-selective lipoxygenase and LT biosynthesis inhibitor resulted in less airway obstruction, as determined by a decrease in airway inspiratory Ptr, compared to the non-blocked (Control) group on both diets. However, the HS diet resulted in increased airway inspiratory Ptr, in both the HS-CON and HS-BLO groups, indicating increased airway obstruction, compared to the NS-CON and NS-BLO groups, respectively.

Bladder urine samples were analyzed for urinary concentration of electrolytes and demonstrated that, while on the HS diet, urinary sodium and chloride were higher (126 and 93.6 mEq/L, respectively) compared to the NS diet (84.9 and 49.4 mEq/L, respectively). There was no change in glomerular filtration rate (creatinine excretion).

While it is recognized that a bladder urine sample is not representative of a two-week dietary protocol, it is indicative of the last meal eaten by the guinea pigs. In combination with the dietary data, the urine analyses confirm the effectiveness of the two diets.

A limitation of this study is that the airway response to hyperpnea challenge was quantified from changes in Ptr, that reflect alterations in respiratory system impedance. The pulmonary response to hyperpnea challenge is clearly complex and involves changes in both airway and tissue resistance (47), as well as microvascular leak (31). However, it is assumed that most of the measured response is a consequence of airway narrowing resulting from airway smooth muscle contraction, which has been confirmed by morphometric studies (47).

This study supports data from prior studies (46, 56, 58) that anesthetized mechanically ventilated guinea pigs develop self-limited bronchoconstriction after dry gas hyperpnea challenge. This response is very reproducible with consecutive challenges. However, qualitative and quantitative differences between the guinea pig response to dry gas hyperpnea and EIA in humans are likely. The oropharynx and larynx in asthmatic subjects provide substantial conditioning of inspired gas delivered to the lower airways. This mechanism is bypassed with the tracheal cannula and small amount of ventilator tubing, as described in the present study. Increasing minute ventilation during hyperpnea presumably increases penetration of the unconditioned gas beyond the trachea into more distal central airways. This has been shown to occur in dogs (59) and in normal (32, 42) or asthmatic human subjects (32); however, inference about how the longitudinally distributed heat and water losses actually change airway wall temperature or hydration (63, 64) is purely speculative. Mechanistic differences in

transduction of the stimulus to bronchoconstriction also could exist, based on species-dependent mediator response, activation of specific neural pathways, and airway smooth muscle anatomy.

Ray et al. (56) have shown that the stimulus for HIAO occurs during, rather than after, dry gas hyperpnea. The magnitude of peak bronchoconstriction depends heavily on three features of the hyperpnea challenge itself: 1) the duration of the hyperpnea challenge, 2) the minute ventilation during dry gas hyperpnea, and 3) the heat and water content of inspired gas during hyperpnea (i.e., bronchoconstriction does not occur when inspired gas is warmed and humidified throughout hyperpnea). Although its magnitude is small, bronchoconstriction is evident after 5 min of dry gas hyperpnea challenge. Furthermore, the absence of bronchoconstriction during 10 min of warm humidified gas hyperpnea indicates that heat and water losses associated with dry gas breathing are the key features responsible for the bronchoconstrction that occurs during dry gas hyperpnea. Finally, a prominent feature of the results of Ray et al. (56) and those of Blackie et al. (8), is that the magnitude of bronchoconstriction increased with the duration of dry gas hyperpnea. It has been suggested that an extended duration of thermal stimulus during dry gas hyperpnea could conceivably result (indirectly or directly) in a greater total release of tachykinins from airway sensory nerves; a pathogenetic step that seems to be critical for the development of HIAO in guinea pigs (30, 31, 37, 40, 57, 62, 69). However, the precise mechanism by which airway heat/losses are transduced into tachykinin release remains unknown.

It is possible that dry gas hyperpnea reversibly impairs airway epithelial cell function in a way that potentiates bronchoconstriction. This might conceivably occur by

1) epithelial dehydration (4), which might facilitate exposure of airway sensory nerve endings, which contains tachykinins substance P (SP) and neurokinin A (NKA) to stimulatory thermal effects of dry gas hyperpnea (29), or 2) inhibition of epithelial neutral endopeptidase (67), which cleaves and inactivates tachykinins. Pharmacological inhibition of this enzyme has been shown to potentiate tachykinin-induced bronchoconstriction (61), including guinea pig HIAO (30, 31, 37, 40, 57, 62, 69).

The respiratory tract in guinea pigs has a rich network of sensory nerve and sensory receptor types containing tachykinins (38). Sensory nerve endings have been identified around vascular and nonvascular smooth muscle, seromucus glands, and penetrating into the surface epithelium (66). There are three main groups of lung receptors: the slowly adapting stretch receptors in airway smooth muscle, the rapidly adapting (irritant) stretch receptors, and the C-fiber receptors located in the epithelium. While the lung receives C-fibers that are of are of spinal origin, the trachea is innervated with C-fibers of vagal origin (66). It has been suggested that tachykinins, which are located in airway afferent nerve endings, are the mediators of HIAO in guinea pigs (37, 57, 62).

Activation of afferent C-fibers causes the release of tachykinins which, in turn, may induce bronchoconstriction, vascular leakage, and mucus secretion (5, 38). Afferent C-fibers can be stimulated by hyperpnea (57) and by other irritants. Tachykinins may directly or indirectly induce airway constriction. In guinea pigs, both NKA and SP are potent bronchoconstrictors (5). In addition, tachykinins can activate mast cells (36), neutrophils (60), and macrophages (10) which, in turn, can release other airway constrictors, such as histamine, LTs, and prostaglandins.

Although, tachykinins appear to be key mediators of HIAO in guinea pigs (31, 62), little evidence exists for their participation in human HIAO: a NK-1 and NK-2 dual tachykinin receptor antagonist (FK-224) inhibits NKA-induced bronchoconstriction in guinea pigs (45), but fails to do so in human asthmatics (35). Although capsaicin reduces HIAO in guinea pigs (29, 70), it does not alter airway responsiveness to hypertonic saline in children with asthma (12). The NK-1 receptor antagonist CP-99,994 inhibits airway response of healthy subjects to SP, but does not protect against hypertonic saline-induced bronchoconstriction in individuals with asthma (23). Finally, FK-888, another NK-1 receptor antagonist, does not inhibit the development of exercise-induced airway obstruction in asthmatic patients [Ichinose, 1996 #672]. Although a different picture may emerge with the advent of new and improved neurokinin antagonists, these studies suggest that tachykinins do not play a prominent role in the development of HIAO in humans.

Given the previously documented (31, 37, 57, 58, 62, 70) critical role of sensory neuropeptides in guinea pig HIAO, one must reconcile how eicosanoid mediators also make an important contribution. When administered exogenously, prostaglandin $PGF_{2\alpha}$, PGD_2, TxA_2 analogue, and the sulfidopeptide LTs are bronchoconstrictors in this species (30). Cysteinyl-LTs, in particular LTD_4, are also known to contribute to HIAO in the guinea pig (13, 30). Dry gas-induced bronchoconstriction in the guinea pig has been shown to be inhibited by the 5-lipoxygenenase (5-LO) inhibitor, A-63162, and the LTD_4 antagonist, ICI-198615 (30). In addition, the LT receptor antagonist, FPL 55712, and LT synthesis inhibitor, MK-886, significantly attenuated HIAO, as well as hyperpnea-induced increases in LT and SP levels. These results suggest that LTs play an important

role in HIAO (37). Increases in plasma and BAL LT levels during the recovery period indicate that hyperventilation causes an increase in LT release. The mechanism for this release is unknown. It could be speculated that hyperventilation-related water and/or heat loss stimulates inflammatory cells to release mediators including LTs, which may directly or indirectly induce airway construction. Leukotrienes are potent airway constrictors (19) and may trigger the release of other bronchoconstrictors, such as tachykinins (9, 40). The completeness of the blockade of HIAO by an LTD_4 antagonist and a 5-lipoxygenase inhibitor provide strong circumstantial evidence that cysteinyl LTs interact with tachykinins to cause airway narrowing (34, 39).

At present it is not clear whether LTs and tachykinins act independently or in combination on airway smooth muscle cells to cause HIAO. Tachykinins have been reported to cause lung mast cell degranulation and pro-inflammatory mediator release in the rat (2), and in human subjects (14). Such a link between tachykinin and secondary release of bronchoconstrictors by airway cells has been confirmed in vivo in the guinea pig: neurokinin antagonists inhibit the synthesis of cysteinyl-LTs evoked by dry gas challenge (70). Yang et al. (70) demonstrated that NK_1 and NK_2 antagonists block both isocapnic dry gas HIAO and observed an increase in biliary LTs, and concluded that LTs are released by tachykinins and are the final mediators of HIAO. However, Lai et al. (37) demonstrated, in guinea pigs, that hyperpnea-induced increases in BAL tachykinin levels were significantly attenuated by LT receptor antagonists FPL 55712 and MK-886. In addition, Garland et al. (30) proposed that eicosanoids modulate HIAO by influencing tachykinin release from afferent C-fibers. Both groups suggest that LTs may trigger the release of tachykinins, which act as the mediators of HIAO. Further studies are needed

to elucidate the sequential release of LTs and tachykinins and their subsequent role in HIAO.

Several potential mechanisms that result in HIAO have been suggested: 1) both airway cooling and subsequent rewarming are necessary for the initiation of HIAO (56), and that airway cooling per se may moderate hyperpnea-induced injury by altering ciliated, goblet, and mast cell membrane fluidity and structure (18). It has been demonstrated that hyperpnea-induced mucosal injury occurs in guinea pigs (33), dogs (27), and asthmatic humans (54), and it is possible that either direct stimulation or damage of the bronchial mucosa may independently initiate HIAO via the release of epithelium-derived mediators; 2) HIAO is mediated by inflammatory mediator release and production, such as LTs and tachykinins (30, 31, 37, 57, 69, 70); and 3) studies in guinea pigs (31) suggest that bronchovascular hyperpermeability develops simultaneously with HIAO and may play a major role in airway narrowing following dry gas hyperpnea. However, morphometric analysis of guinea pig lungs have shown that smooth muscle contraction, and not airway edema, is responsible for HIAO (47). It is possible that bronchovascular leakage may provide water for the maintenance of mucosal hydration and mucociliary clearance during and after hyperpnea. The fact that hypertonic saline and hyperpnea with dry air enhances mucociliary clearance in normal and asthmatic subjects (16, 17) support this hypothesis. Instead of contributing to the development of HIAO, microvascular leakage appears to protect the airway mucosa from hyperpnea-induced injury.

The most effective inhibitors of HIAO are β_2-adrenoreceptor agonists (69), which act primarily by reducing smooth muscle responsiveness. Their efficacy in protecting

against HIAO and the speed with which HIAO develops and subsides (69) strongly support the hypothesis that mediator-induced smooth muscle constriction causes HIAO. Conversely, McFadden et al. (43) speculated that airway cooling transiently decreased bronchial blood flow and that rewarming resulted in bronchovascular hyperemia, engorgement, and airway edema that resulted in airway obstruction. However, bronchial blood flow in dogs (3) and sheep (51) increases during hyperventilation with dry air. Although, these data do not support the hypothesis that cooling induces bronchovascular constriction, they do not rule out the possibility that airway hyperemia and edema contribute to the development of HIAO (43). Therefore, although HIAO primarily results from airway smooth muscle constriction, airway edema may enhance its effect.

The potential mechanisms that contribute to the development of HIAO are clearly complex. The mechanism by which dietary NaCl loading augments the development of HIAO in guinea pigs is unknown. It has been that shown that dietary NaCl loading decreases intracellular pH causing a metabolic acidosis, and increases intracellular sodium $[Na^+]_i$ and intracellular calcium $[Ca^{2+}]_i$ levels via inhibition of the Na^+ - Ca^{2+} exchanger, in rat peritoneal mast cells, resulting in histamine release (55). Leukotriene release was not evaluated, but it is likely that LTs are also released from effector cells in the airways (30). Dumitriu et al. (20) demonstrated that LTD_4 acting on specific receptors increases $[Ca^{2+}]_i$ resulting in contraction of guinea pig tracheal smooth muscle, while Oliva et al. (49) observed the same effect in guinea pig ileal longitudinal smooth muscle .

An elevation in dietary NaCl may have a profound effect on blood volume in the bronchial circulation and hence on airway diameter. An increase in vascular volume and microvascular pressure may lead to mucosal edema and hence a narrowing of the airway lumen (41). This could augment the effects of smooth muscle contraction. In addition, it has been shown that high dietary sodium loads inhibits the Na+/K+ ATPase in erythrocytes of normotensive males (68) and asthmatic subjects (52). The resulting inhibition would be expected to increase $[Na^+]_i$, thereby raising $[Ca^{2+}]_i$ levels via inhibition of the Na^+ - Ca^{2+} exchanger, thereby resulting in smooth muscle contraction (52, 65), as well as enhancing the release of inflammatory mediators (11).

It is possible that changes in NaCl may act upstream of mediator release by directly effecting airway osmolarity (initiating stimulus of EIA). The stimulation of sensory nerves by airway osmolarity is established in animal models of EIA (53), but evidence that sensory neuropeptides are directly involved in human EIA is lacking. The development of specific peptide antagonists for study in EIA is eagerly awaited.

In conclusion, while the mechanism of how dietary NaCl loading augments the development of HIAO in guinea pigs is unknown, this study has clearly demonstrated that increasing NaCl consumption in guinea pigs results in increased airway obstruction in both the HS-CON and HS-BLO groups compared to the NS group, and suggests that LT release can be influenced by changes in dietary NaCl loading. Further studies are needed to elucidate a mechanistic pathway by which NaCl effects LT release from mediator cells and/or tachykinin release from afferent sensory C-fibers in the airways, and whether LTs and tachykinins act independently or in combination on airway smooth muscle to cause HIAO. It is possible, however, that dietary NaCl loading acts

via changing airway osmolarity and the subsequent release of mediators such as LTs, resulting in airway smooth muscle contraction.

References

1. Arm, J. P., C. E. Horton, F. House, T. J. Clark, B. W. Spur, and T. H. Lee. Enhanced generation of leukotriene B4 by neutrophils stimulated by unopsonized zymosan and by calcium ionophore after exercise-induced asthma. *Am Rev Respir Dis* 138: 47-53, 1988.

2. Assem, E. S., N. S. Ghanem, N. A. Abdulah, and H. Repke. Subsatnce P and arg-pro-lys-pro-c12-h25-induced mediator release from different mast cell subtypes of rat and guinea pig. *Immunopharmacology* 17: 119-128, 1989.

3. Baile, E. M., R. W. Dahlby, B. R. Wiggs, G. H. Parsons, and P. D. Pare. Effect of cold and warm dry air hyperventilation on canine airway blood flow. *J Appl Physiol* 62: 526-532, 1987.

4. Barbet, J. P., M. Chauveau, S. Labbe, and A. Lockhart. Breathing dry air causes acute epithelial damage and inflammation of the guinea pig trachea. *J Appl Physiol* 64: 1851-1857, 1988.

5. Barnes, P. J., J. N. Baraniuk, and M. G. Belvisi. Neuropeptides in the respiratory tract. *Am Rev Respir Dis* 144: 1187-1198, 1991.

6. Beck, K. C., K. P. Offord, and P. D. Scanlon. Bronchoconstriction occurring during exercise in asthmatic subjects. *Am J Respir Crit Care Med* 149: 352-7, 1994.

7. Biagini, R. E., J. C. Clark, W. J. Moorman, and E. A. Knecht. Evaluation of the onset and duaration of response to cold air inhalation challenge in cynomolgus monkeys (Macaca fascicularis). *J Appl Toxicol* 11: 1-6, 1991.

8. Blackie, S. P., C. Hilliam, R. Village, and P. D. Pare. The time course of bronchoconstriction in asthmatics during and after isocapnic hyperventilation. *Am Rev Respir Dis* 142: 1133-6, 1990.

9. Bloomquist, E. I., and R. M. Kream. Release of substance P from guinea pig trachea leukotriene D4. *Exp Lung Res* 16: 645-59, 1990.

10. Brunelleschi, S., S. Guidotto, I. Viano, R. Fantozzi, E. Pozzi, P. Ghio, and C. Albera. Tachykinin activation of human alveolar macrophages in tobacco smoke and sarcoidosis: a phenotypical and functional study. *Neuropeptides* 30: 456-64, 1996.

11. Cabado, A. G., M. R. Vieytes, and L. M. Botana. Inhibition of Na+/K+ ATPase under hypertonic conditions in rat mast cells. *Life Sci* 63: 1227-37, 1998.

12. Chang, A. B., P. D. Phelan, D. Holst, and C. F. Robertson. Effect of capsaicin on airway responsiveness to hypertonic saline challenge in asthmatic and non-asthmatic children. *Pediatr Pulmonol* 23: 412-6, 1997.

13. Chapman, R. W., and G. Danko. Hyperventilation-induced bronchoconstriction in guinea pigs. *Int Arch Allergy Appl Immunol* 78: 190-6, 1985.

14. Cross, L. J., L. G. Heaney, and M. Ennis. Histamine release from bronchoalveolar lavage mast cells by neurokinin A and bradykinin. *Inflamm Res* 46: 306-309, 1997.

15. Dahlen, S. E. Pulmonary effects of leukotrienes. *Acta Physiol Scand Suppl* 512: 1-51, 1983.

16. Daviskas, E., S. D. Anderson, and I. Gonda. Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. *Eur J Respir J* 9: 725-732, 1996.

17. Daviskas, E., S. D. Anderson, I. Gonda, H. K. Chan, P. Cook, and R. Fulton. Changes in mucociliary clearance during and after isocapnic hyperventilation in asthmatic and healthy subjects. *Eur Respir J* 8: 742-751, 1995.

18. DeKruijff, B., P. R. Cullis, A. J. Verkleij, M. H. Hope, C. J. A. Van Echteld, and T. F. Taraschi. Lipid polymorphism and membrane function. In: *The enzymes of biological membranes*, edited by A. N. Martonosi. New York: Plenus Press, 1985, p. 131-204.

19. Drazen, J. M. Leukotrienes as mediators of airway obstruction. *Am J Respir Crit Care Med* 158: S193-200, 1998.

20. Dumitriu, D., S. Prie, S. G. Bernier, G. Guillemette, and P. Sirois. Mechanism of action of leukotriene D4 on guinea pig smooth muscle cells: roles of Ca++ influx and intracellular Ca++ release. *J Pharmacol Exp Ther* 280: 1357-65, 1997.

21. Eggleston, P. A., A. Kagey-Sobotka, R. P. Schleimer, and L. M. Lichtenstein. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 130: 86-91, 1984.

22. Ellis, J. L., and B. J. Undem. Role of peptidoleukotrienes in capsaicin-sensitive sensory fibre- mediated responses in guinea-pig airways. *J Physiol (Lond)* 436: 469-84, 1991.

23. Fahy, J. V., H. H. Wong, P. Geppetti, J. M. Reis, S. C. Harris, D. B. Maclean, J. A. Nadel, and H. A. Boushey. Effect of an NK1 receptor antagonist (CP-99,994) on hypertonic saline-induced bronchoconstriction and cough in male asthmatic subjects. *Am J Respir Crit Care Med* 152: 879-84, 1995.

24. Finnerty, J. P., A. Harvey, and S. T. Holgate. The relative contributions of histamine and prostanoids to bronchoconstriction provoked by isocapnic hyperventilation in asthma. *Eur Respir J* 5: 323-30, 1992.

25. Freed, A. N., and M. S. Davis. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. *Am J Respir Crit Care Med* 159: 1101-7, 1999.

26. Freed, A. N., L. J. Kelly, and H. A. Menkens. Airflow-induced bronchospasm: imbalance between airway cooling and airway drying. *Am Rev Respir Dis* 136: 595-599, 1987.

27. Freed, A. N., S. P. Peters, and H. A. Menkes. Airflow-induced bronchoconstriction: role of epithelium and eicosanoid mediators. *J Appl Physiol* 62: 574-81, 1987.

28. Freed, A. N., D. Wang, and H. A. Menkes. Dry air-induced constriction: effects of pharmacological intervention and temperature. *J Appl Physiol* 62: 1794-800, 1987.

29. Frossard, N., K. J. Rhoden, and P. J. Barnes. Influence of epithelium on guinea pig airway responses to tachykinins: role of endopeptidase and cyclooxygenase. *J Pharmacol Exp Ther* 248: 292-8, 1989.

30. Garland, A., J. E. Jordan, D. W. Ray, S. M. Spaethe, L. Alger, and J. Solway. Role of eicosanoids in hyperpnea-induced airway responses in guinea pigs. *J Appl Physiol* 75: 2797-804, 1993.

31. Garland, A., D. W. Ray, C. M. Doerschuk, L. Alger, S. Eappon, C. Hernandez, M. Jackson, and J. Solway. Role of tachykinins in hyperpnea-induced bronchovascular hyperpermeability in guinea pigs. *J Appl Physiol* 70: 27-35, 1991.

32. Gilbert, I. A., K. A. Lenner, J. M. Fouke, A. J. Coreno, and E. R. McFadden. Comparison of intrathoracic temperatures during exercise and recovery and in normal and asthmatic subjects. *Physiologist* 29: 143, 1986.

33. Ingenito, E. P., L. B. Pliss, R. H. Ingram, Jr., and B. M. Pichurko. Bronchoalveolar lavage cell and mediator responses to hyperpnea-induced bronchoconstriction in the guinea pig. *Am Rev Respir Dis* 141: 1162-6, 1990.

34. Israel, E., R. Dermarkarian, M. Rosenberg, R. Sperling, G. Taylor, P. Rubin, and J. M. Drazen. The effects of a 5-lipoxygenase inhibitor on asthma induced by cold, dry air [see comments]. *N Engl J Med* 323: 1740-4, 1990.

35. Joos, G. F., J. Van Schoor, J. C. Kips, and R. A. Pauwels. The effect of inhaled FK224, a tachykinin NK-1 and NK-2 receptor antagonist, on neurokinin A-induced bronchoconstriction in asthmatics. *Am J Respir Crit Care Med* 153: 1781-4, 1996.

36. Krumins, S. A., and C. A. Broomfield. C-terminal substance P fragments elicit histamine release from a murine mast cell line. *Neuropeptides* 24: 5-10, 1993.

37. Lai, Y. L., and S. P. Lee. Mediators in hyperpnea-induced bronchoconstriction of guinea pigs. *Naunyn Schmiedebergs Arch Pharmacol* 360: 597-602, 1999.

38. Lundberg, J. M., K. Alving, J. A. Karlsson, R. Matran, and G. Nilsson. Sensory neuropeptide involvement in animal models of airway irritation and of allergen-evoked asthma. *Am Rev Respir Dis* 143: 1429-30; discussion 1430-1, 1991.

39. Manning, P. J., R. M. Watson, D. J. Margolskee, V. C. Williams, J. I. Schwartz, and P. M. O'Byrne. Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D4-receptor antagonist [see comments]. *N Engl J Med* 323: 1736-9, 1990.

40. Martins, M. A., S. A. Shore, and J. M. Drazen. Release of tachykinins by histamine, methacholine, PAF, LTD4, and substance P from guinea pig lungs. *Am J Physiol* 261: L449-55, 1991.

41. McFadden, E. R., Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon [see comments]. *Lancet* 335: 880-3, 1990.

42. McFadden, E. R., Jr., D. M. Denison, J. F. Waller, B. Assoufi, A. Peacock, and T. Sopwith. Direct recordings of the temperatures in the tracheobronchial tree in normal man. *J Clin Invest* 69: 700-5, 1982.

43. McFadden, E. R., K. A. M. Lenner, and K. P. Strohl. Postexertional airway rewarming and thermally induced asthma. *J Clin Invest* 78: 18-25, 1986.

44. McFadden, E. R., Jr., J. A. Nelson, M. E. Skowronski, and K. A. Lenner. Thermally induced asthma and airway drying. *Am J Respir Crit Care Med* 160: 221-6, 1999.

45. Murai, M., H. Morimoto, Y. Maeda, S. Kiyotoh, M. Nishikawa, and T. Fujii. Effects of FK224, a novel compound NK1 and NK2 receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory nerve stimulation in guinea pigs. *J Pharmacol Exp Therapeutics* 262: 403-408, 1992.

46. Murphy, T. M., D. W. Ray, L. E. Alger, I. J. Phillips, J. C. Roach, A. R. Leff, and J. Solway. Ontogeny of dry gas hyperpnea-induced bronchoconstriction in guinea pigs. *J Appl Physiol* 76: 1150-5, 1994.

47. Nagase, T., M. J. Dallaire, and M. S. Ludwig. Airway and tissue responses during hyperpnea-induced constriction in guinea pigs. *Am J Respir Crit Care Med* 149: 1342-7, 1994.

48. Ohtsuka, A., S. Koyama, and T. Horie. Refractoriness of eucapnic hyperventilation-induced bronchoconstriction in rabbits. *Am J Respir Crit Care Med* 149: 682-686, 1994.

49. Oliva, D., M. R. Accomazzo, S. Giovanazzi, and S. Nicosia. Correlation between leukotriene D4-induced contraction and cytosolic calcium elevation: a quantitative and simultaneous evaluation in smooth muscle. *J Pharmacol Exp Ther* 268: 159-66, 1994.

50. Omori, C., S. B. H., W. Mitzner, and A. N. Freed. Hyperpnea with dry air causes time-dependent alterations in mucosal morphology and bronchovascular permeability. *J Appl Physiol* 78: 1043-1051, 1995.

51. Parsons, G. H., and P. D. Pare. Airway blood flow response to eucapnic dry air hyperventilation in sheep. *J Appl Physiol* 66: 1443-1447, 1989.

52. Pavord, I. D., S. Dutt, O. J. Carey, D. Spence, S. Brand, and R. F. Bing. Red blood cell sodium transport in asthma. *Thorax* 45: 799, 1990.

53. Pisarri, T. E., A. Jonson, H. M. Coleridge, and J. C. G. Coleridge. Vagal afferent and reflex responses to changes in surface osmolarity in lower airways in dogs. *J Appl Physiol* 73: 2305-2313, 1992.

54. Pliss, L. B., E. P. Ingenito, R. H. Ingram, Jr., and B. Pichurko. Assessment of bronchoalveolar cell and mediator response to isocapnic hyperpnea in asthma. *Am Rev Respir Dis* 142: 73-8, 1990.

55. Praetorius, H. A., U. G. Friis, J. Praetorius, and T. Johansen. Evidence for a Na+/Ca2+ exchange mechanism in rat peritoneal mast cells. *Pflugers Arch* 437: 86-93, 1998.

56. Ray, D. W., A. Garland, C. Hernandez, S. Eappen, L. Alger, and J. Solway. Time course of bronchoconstriction induced by dry gas hyperpnea in guinea pigs. *J Appl Physiol* 70: 504-10, 1991.

57. Ray, D. W., C. Hernandez, A. R. Leff, J. M. Drazen, and J. Solway. Tachykinins mediate bronchoconstriction elicited by isocapnic hyperpnea in guinea pigs. *J Appl Physiol* 66: 1108-12, 1989.

58. Ray, D. W., C. Hernandez, N. Munoz, A. R. Leff, and J. Solway. Bronchoconstriction elicited by isocapnic hyperpnea in guinea pigs. *J Appl Physiol* 65: 934-9, 1988.

59. Ray, D. W., M. Strek, E. P. Ingenito, P. Schumacker, and K. P. Strohl. Respiratory heat loss shifts distally during cold air hyperpnea. *Federation Proc* 46: 511, 1987.

60. Serra, M. C., F. Bazzoni, V. Della Bianca, M. Greskowiak, and F. Rossi. Activation of human neutrophils by substance P. Effect on oxidative metabolism, exocytosis, cytosolic Ca2+ concentration and inositol phosphate formation. *J Immunol* 141: 2118-24, 1988.

61. Shore, S. A., N. P. Stimler-Gerard, S. R. Coats, and J. M. Drazen. Substance P-induced bronchoconstriction in the guinea pig. Enhancement by inhibitors of neutral metalloendopeptidase and angiotensin- converting enzyme. *Am Rev Respir Dis* 137: 331-6, 1988.

62. Solway, J., B. M. Kao, J. E. Jordan, B. Gitter, I. W. Rodger, J. J. Howbert, L. E. Alger, J. Necheles, A. R. Leff, and A. Garland. Tachykinin receptor antagonists inhibit hyperpnea-induced bronchoconstriction in guinea pigs. *J Clin Invest* 92: 315-23, 1993.

63. Solway, J., A. R. Leff, I. Dreshaj, N. M. Munoz, E. P. Ingenito, D. Michaels, R. H. Ingram, Jr., and J. M. Drazen. Circulatory heat sources for canine respiratory heat exchange. *J Clin Invest* 78: 1015-9, 1986.

64. Solway, J., B. M. Pichurko, E. P. Ingenito, E. R. McFadden, Jr., C. H. Fanta, R. H. Ingram, Jr., and J. M. Drazen. Breathing pattern affects airway wall temperature during cold air hyperpnea in humans. *Am Rev Respir Dis* 132: 853-7, 1985.

65. Souhrada, M., and J. F. Souhrada. Immunologically induced alterations of airway smooth muscle cell membrane. *Science* 225: 723-725, 1984.

66. Spina, D., and C. P. Page. Airway sensory nerves in asthma--targets for therapy? *Pulm Pharmacol* 9: 1-18, 1996.

67. Stimler-Gerard, N. P. Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung. *J Clin Invest* 79: 1819-1825, 1987.

68. Weissberg, P. L., M. J. West, M. J. Kendall, M. Ingram, and K. L. Woods. Effect of changes in dietary sodium and potassium on blood pressure and cellular electrolyte handling in young normotensive subjects. *J Hypertens* 3: 475-80, 1985.

69. Yang, X. X., G. Ho, L. J. Xu, W. S. Powell, and J. G. Martin. The beta(2)-agonist salbutamol inhibits bronchoconstriction and leukotriene D(4) synthesis after dry gas hyperpnea in the guinea-pig. *Pulm Pharmacol Ther* 12: 325-9, 1999.

70. Yang, X. X., W. S. Powell, M. Hojo, and J. G. Martin. Hyperpnea-induced bronchoconstriction is dependent on tachykinin- induced cysteinyl leukotriene synthesis. *J Appl Physiol* 82: 538-44, 1997.

Table V-1. Electrolyte content of bladder urine, food consumption and starting and ending weights after 14 days.

Diet	Food consumption (g/day)	Body weight (g)		Bladder urine electrolyte concentration			
		Start of study	End of study	Na^+ (mEq/L)	Cl^- (mEq/L)	K^+ (mEq/L)	Cr (mg/dL)
Normal salt (0.75%)	34.4 ± 0.08	393.3 ± 20.6	532.3 ± 16.9	84.9 ± 6.8	49.4 ± 8.0	126.1 ± 14.5	28.7 ± 2.6
High salt (2%)	32.6 ± 0.43 *	373.4 ± 28.8	517.2 ± 20.9	126.8 ± 11.1†	93.6 ± 15.9†	99.5 ± 10.8	25.8 ± 2.6

Values are means ± SEM; n = 16 measurement in each group. *$p<0.001$, † $p<0.05$, compared to the "normal salt" group. Na^+ = sodium, Cl^- = chloride, K^+ = potassium, and Cr = creatinine

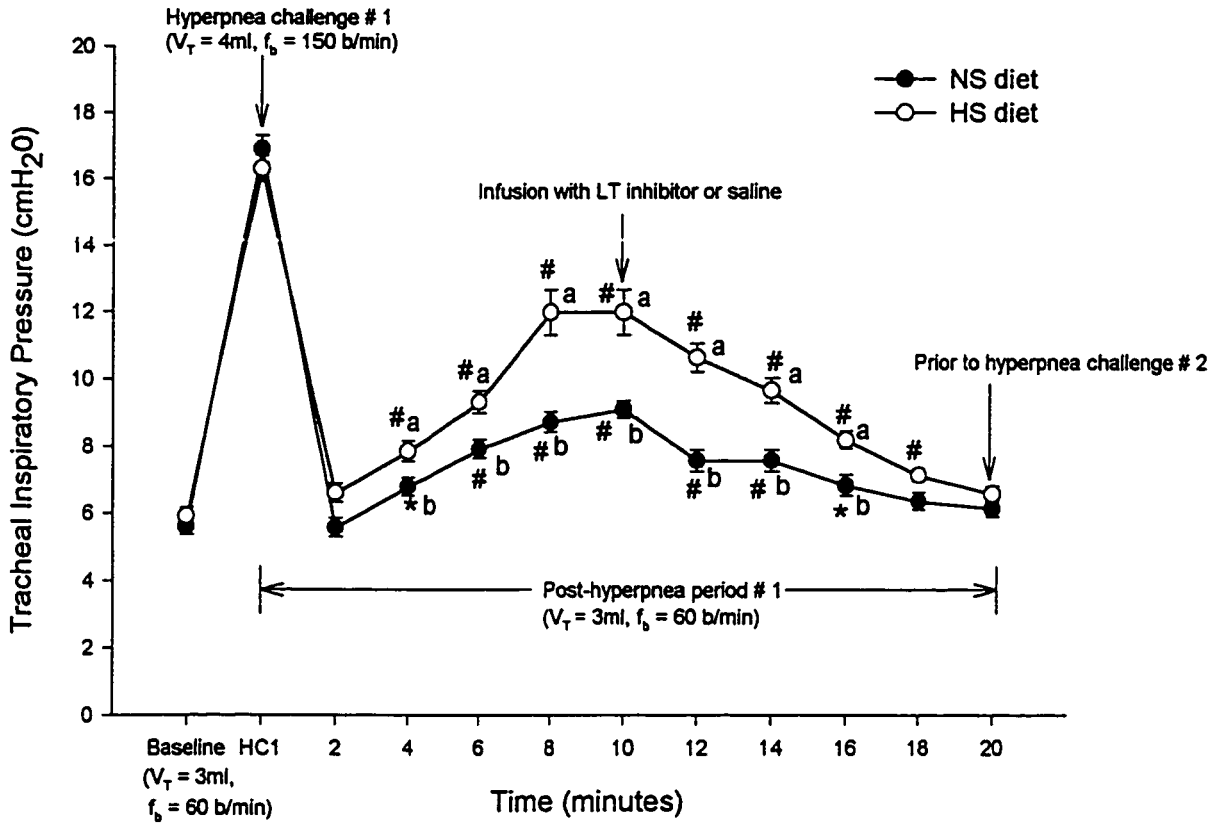


Figure V-1. Time course of inspiratory tracheal pressure (Ptr) measured before (Baseline), during (HC1), and after dry gas hypernea challenge # 1. Values are means ± SEM. Letters (a,b) denote significant difference between diet within time; $p<0.05$ for 4 and 6 min and $p<0.001$ for 8, 10, 12, 14, and 16 min post challenge. # $p<0.001$ and *$p<0.05$ significantly different from baseline within respective diet.

NOTE: When error bars are not evident for any data point in this or subesquent figures, it is because the SEM was so small that error bars fell within the size of the symbol used to plot mean value.

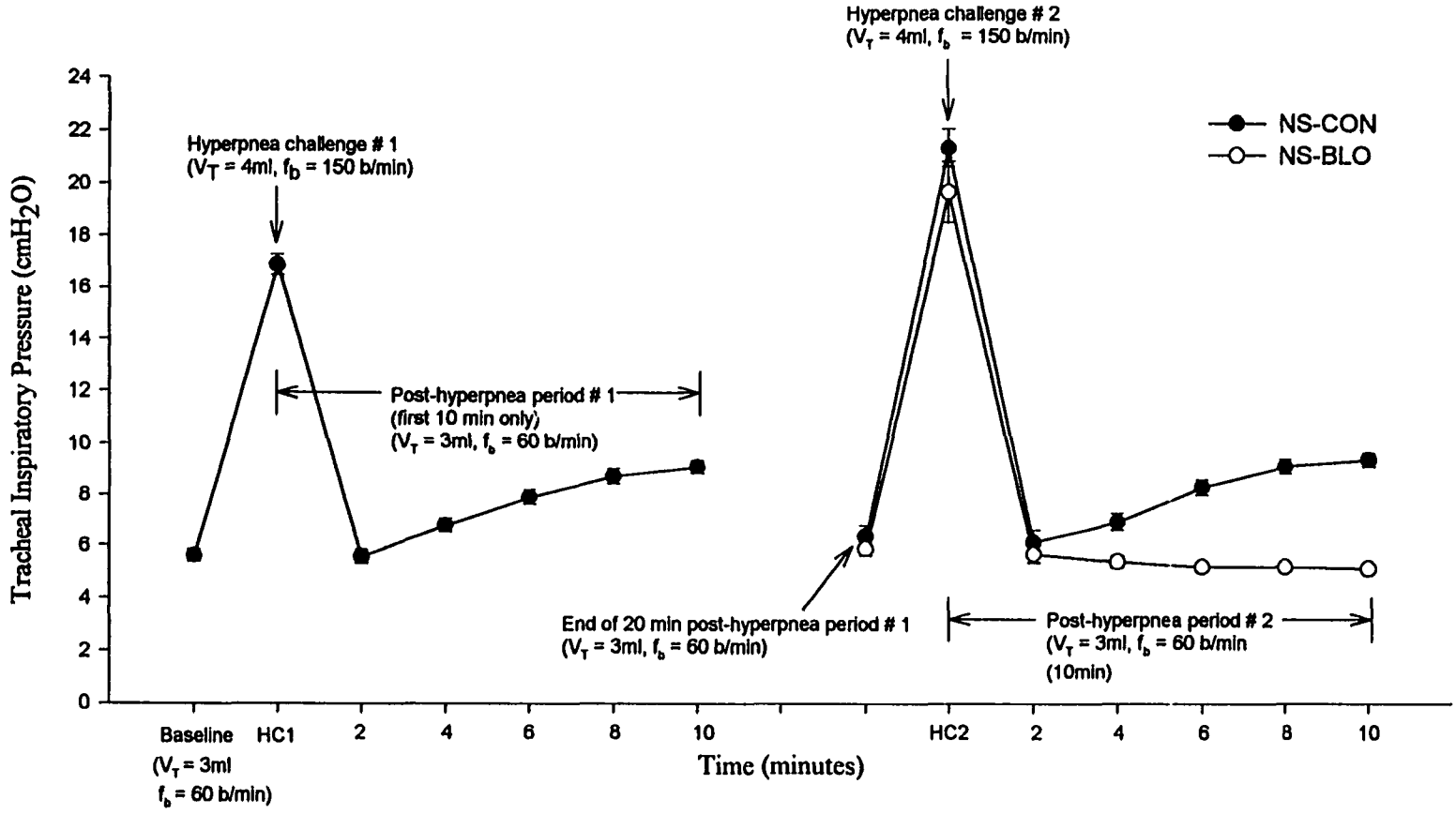


Figure V-2. Time course of inspiratory tracheal pressure (Ptr) in reponse to hyperpnea challenge # 1 and # 2 for NS diet. Values are means ± SEM. Significant difference between dry gas hyperpnea challenges (HC) # 1 and # 2 within diets ($p<0.05$). No significant difference ($p>0.05$) between NS diet (prior to HC2) and NS-CON within respective time periods. Significant difference between NS-BLO and NS diet and NS-BLO and NS-CON, within respective time periods ($p<0.05$ at 4 min, and $p<0.001$ at 6, 8, 10 min).

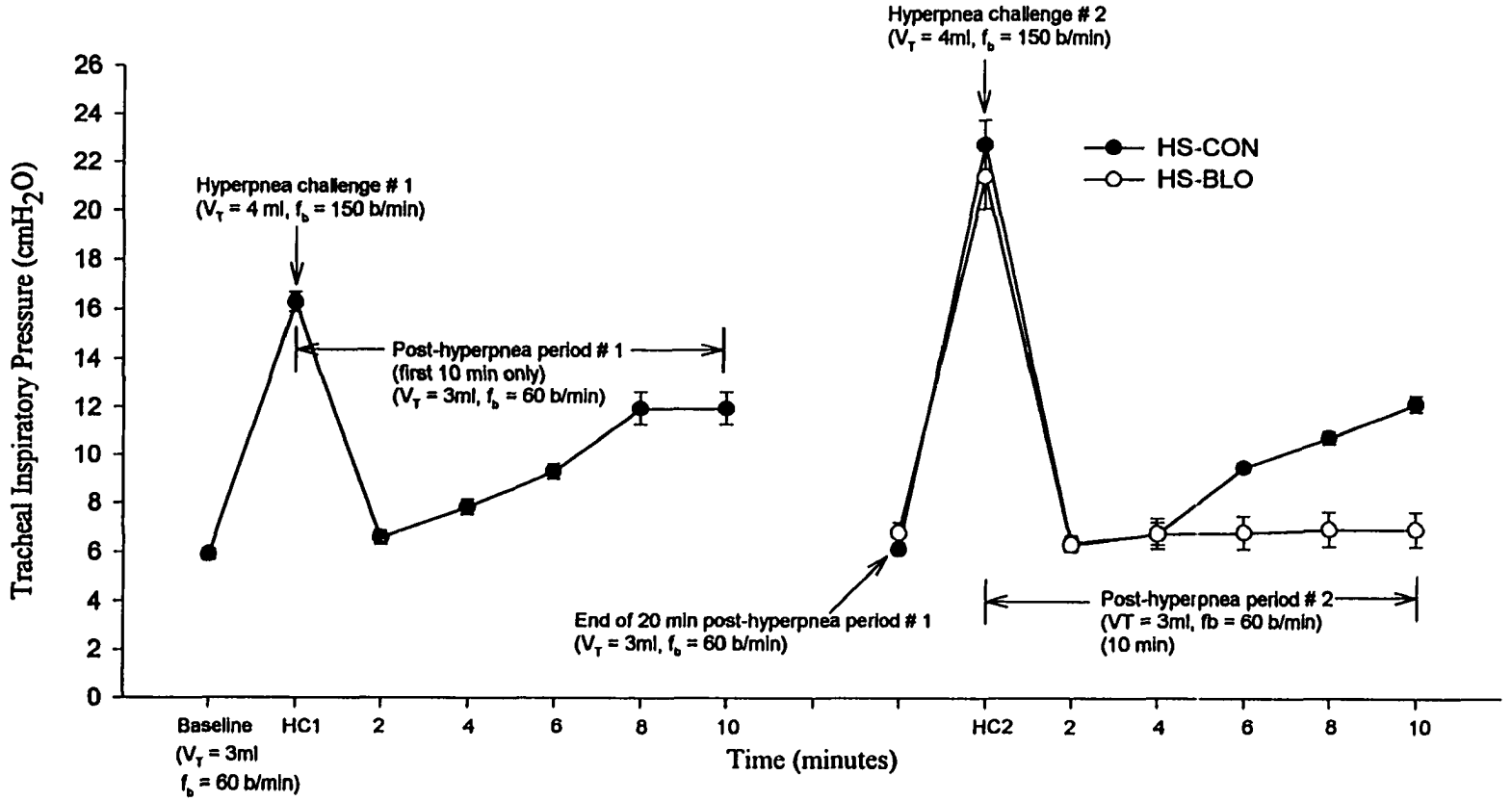


Figure V-3. Time course of inspiratory tracheal pressure (Ptr) in response to hyperpnea challenge # 1 and # 2 for HS diet. Values are means ± SEM. Significant difference between dry gas hyperpnea challenges # 1 and # 2 within diets ($p<0.05$). No significant difference ($p>0.05$) between HS diet (prior to HC2) and HS-CON within respective time periods. Significant difference between HS-BLO and HS-diet and HS-BLO and HS-CON, within respective time periods ($p<0.001$ at 6, 8, and 10 min).

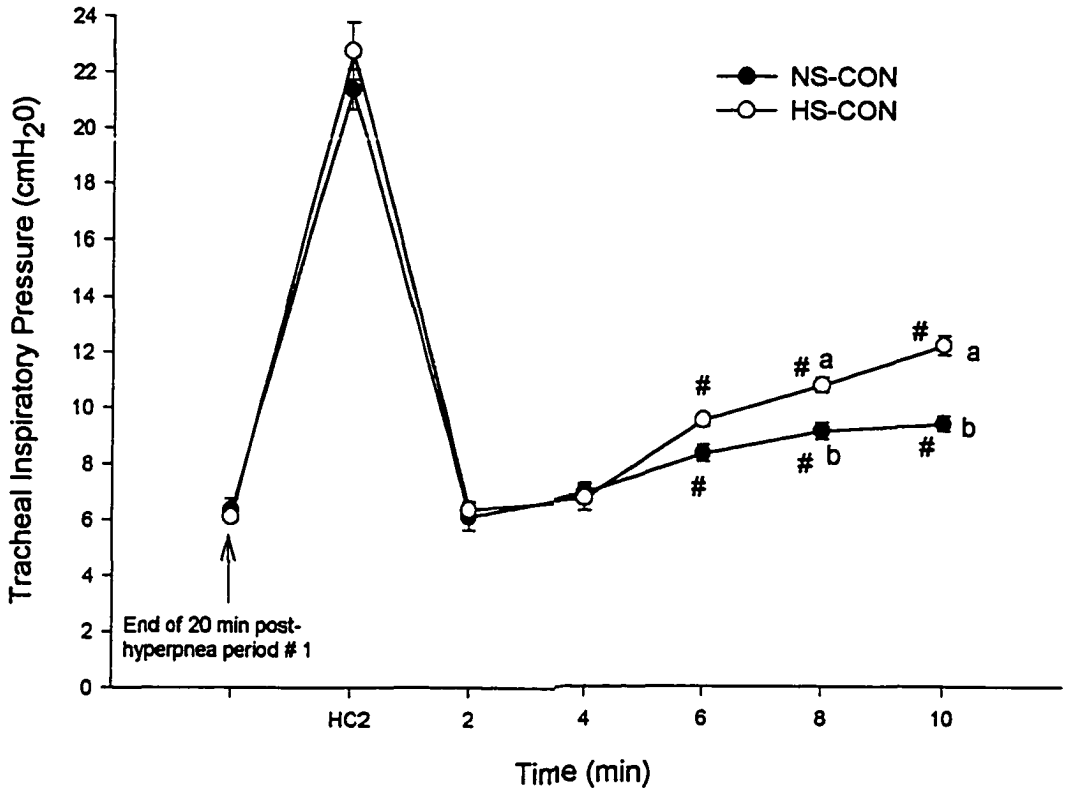


Figure V-4. Time course of inspiratory tracheal pressure (Ptr) for CON groups in response to dry gas hyperpenea challenge # 2. Values are means ± SEM. Letters (a,b) denote significant difference between diets within time; $p<0.05$ for 8 min and $p<0.001$ for 10 min. # $p<0.001$ significantly different to pre-challenge value within respective diet.

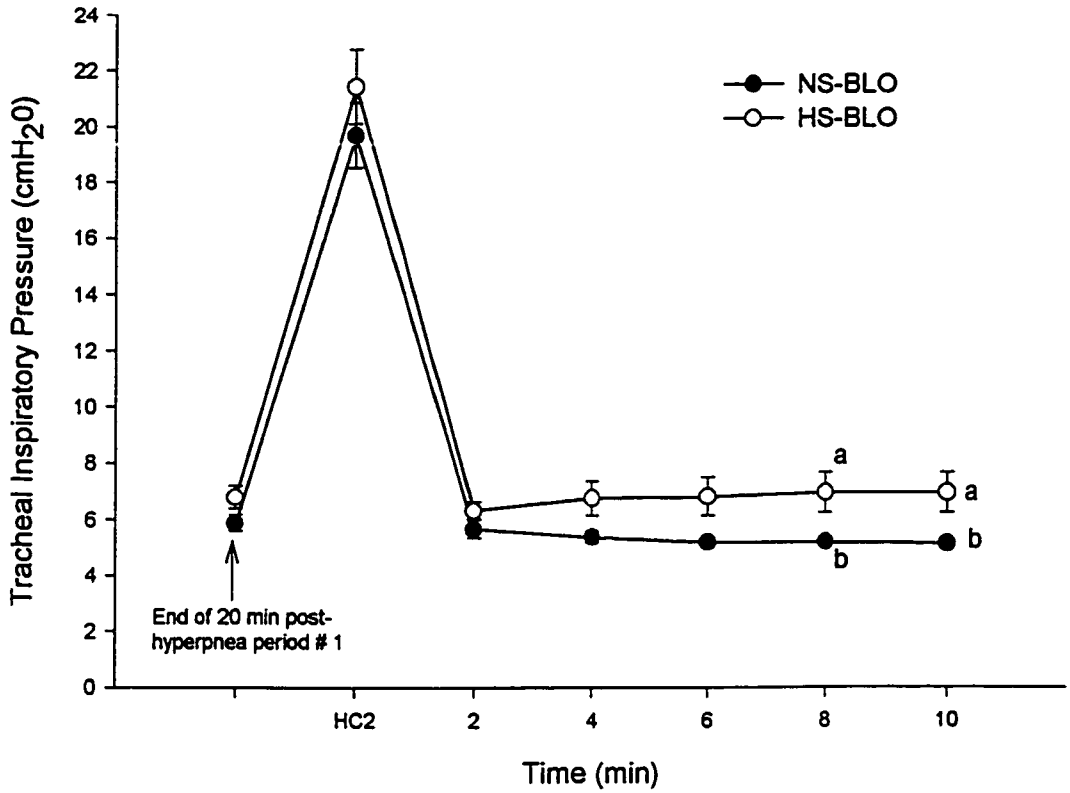


Figure V-5. Time course of inspiratory tracheal pressure (Ptr) for BLO groups in response to dry gas hyperpnea challenge # 2. Values are means ± SEM. Letters (a, b) denote significance between diets within time, $p<0.05$. No significant difference ($p>0.05$) between post-hyperpnea challenge and pre-challenge values within respective diet.

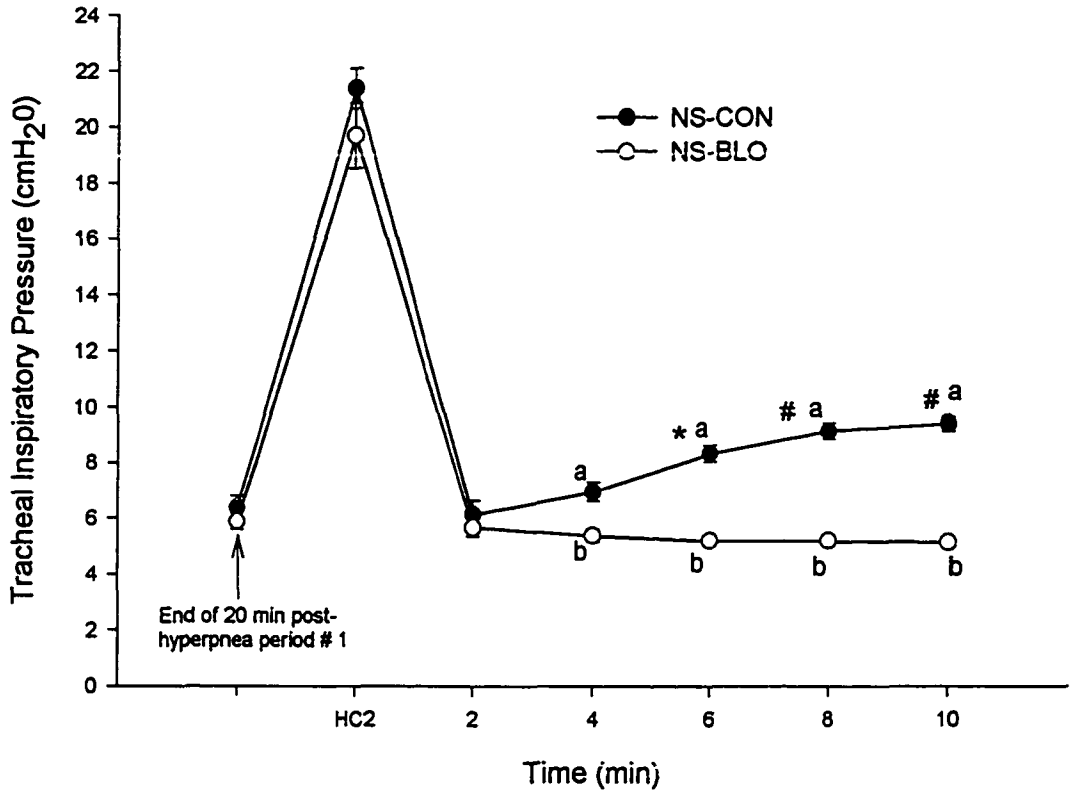


Figure V-6. Time course of inspiratory tracheal pressure for NS-CON and NS-BLO groups in response to dry gas hyperpnea challenge # 2. Values are means ± SEM. Letters (a,b) denote significance between groups within time; $p<0.05$ for 4 min and $p<0.001$ for 6, 8, and 10 min. # $p<0.001$ and * $p<0.05$ significantly different to pre-challenge value within respective group.

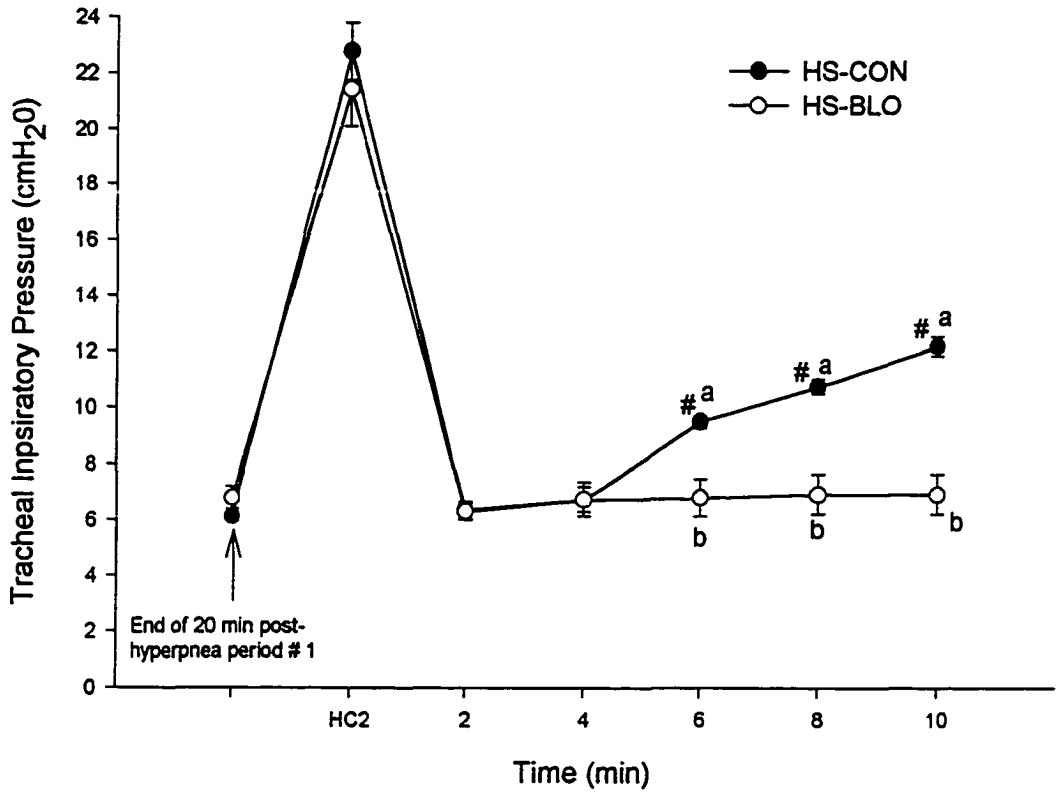


Figure V-7. Time course of inspiratory pressure (Ptr) for HS-CON and HS-BLO groups in response to dry gas hyperpnea challenge # 2. Values are means ± SEM. Letters (a,b) denote significance between groups within time, $p<0.001$. # $p<0.001$ significantly different to pre-challenge value within respective group

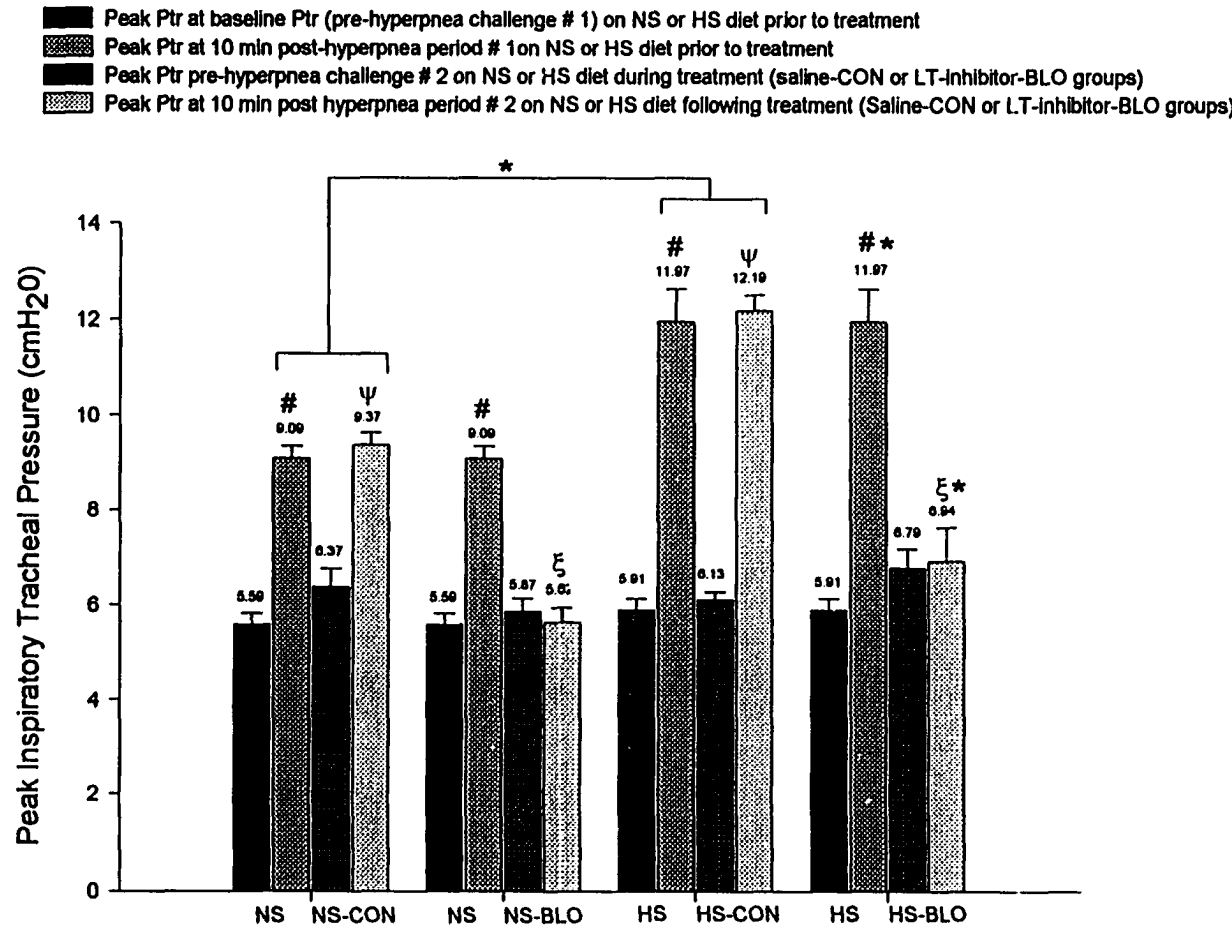


Figure V-8. Peak inspiratory value of Ptr pre- and post hyperpnea challenge # 1 and # 2. Values are mean ± SEM. Significant difference between pre- and post-hyperpnea challenge # 1, within each group (#, $p<0.001$). Significant difference between pre- and post-hyperpnea challenge # 2, within CON groups (ψ, $p<0.001$). Significant difference between post-hyperpnea challenge # 1 and # 2 (ξ, $p<0.001$), within BLO groups. Significant difference between post-hyperpnea challenge # 1 and # 2 resp.(*, $p<0.001$).

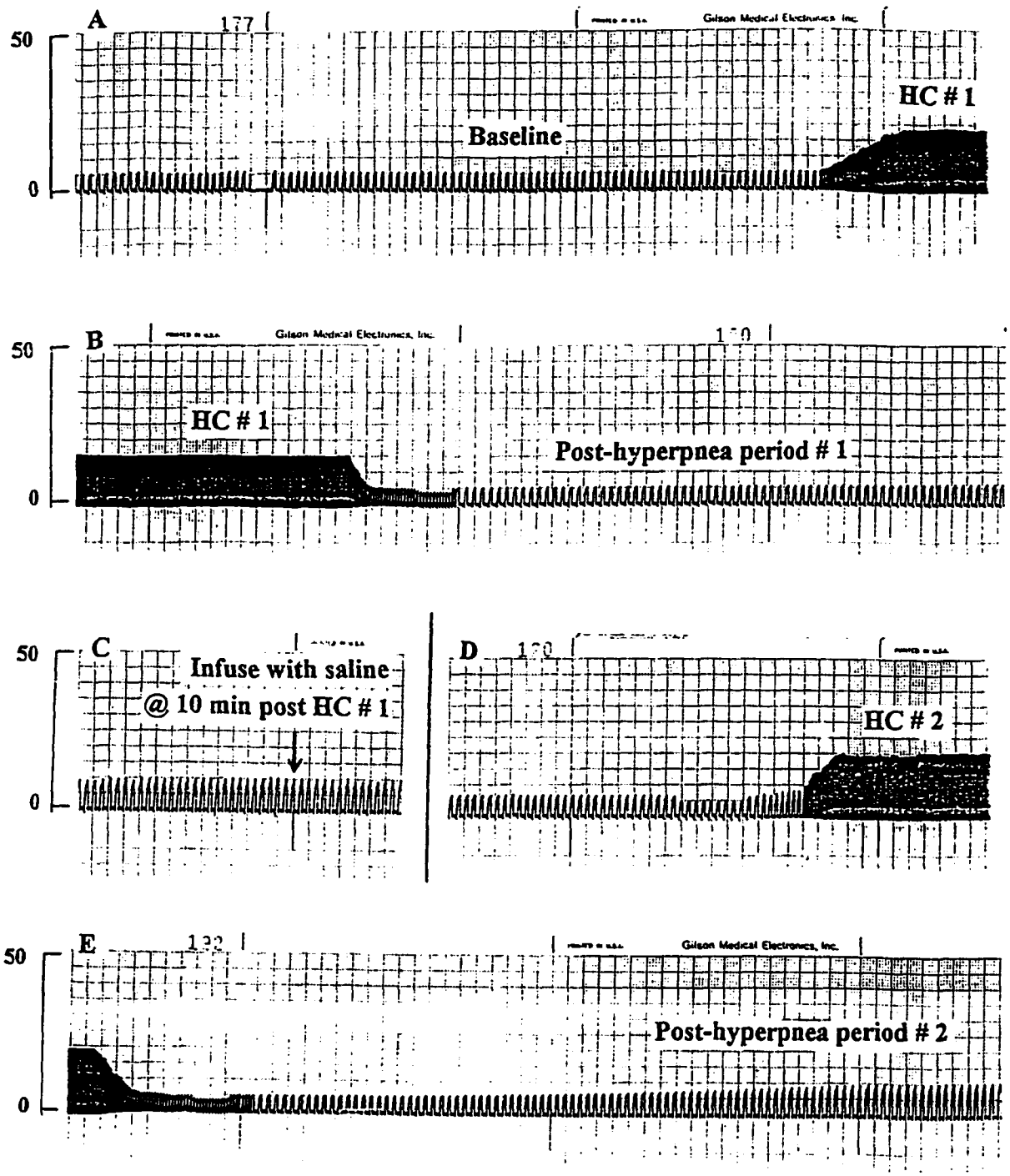


Figure V-9. Example of airway inspiratory pressures for an NSD-CON animal.

A, Baseline and hyperpnea challenge # 1 (HC # 1). B, HC # 1 and post-hyperpnea period # 1. C, Infusion of saline at 10 min post-HC # 1. D, End of post-hyperpnea period # 1 and HC # 2. E, Post-hyperpnea period # 2 . It is evident that HIAO is enhanced during the first 10 min of post-hyperpnea period # 1 and # 2.

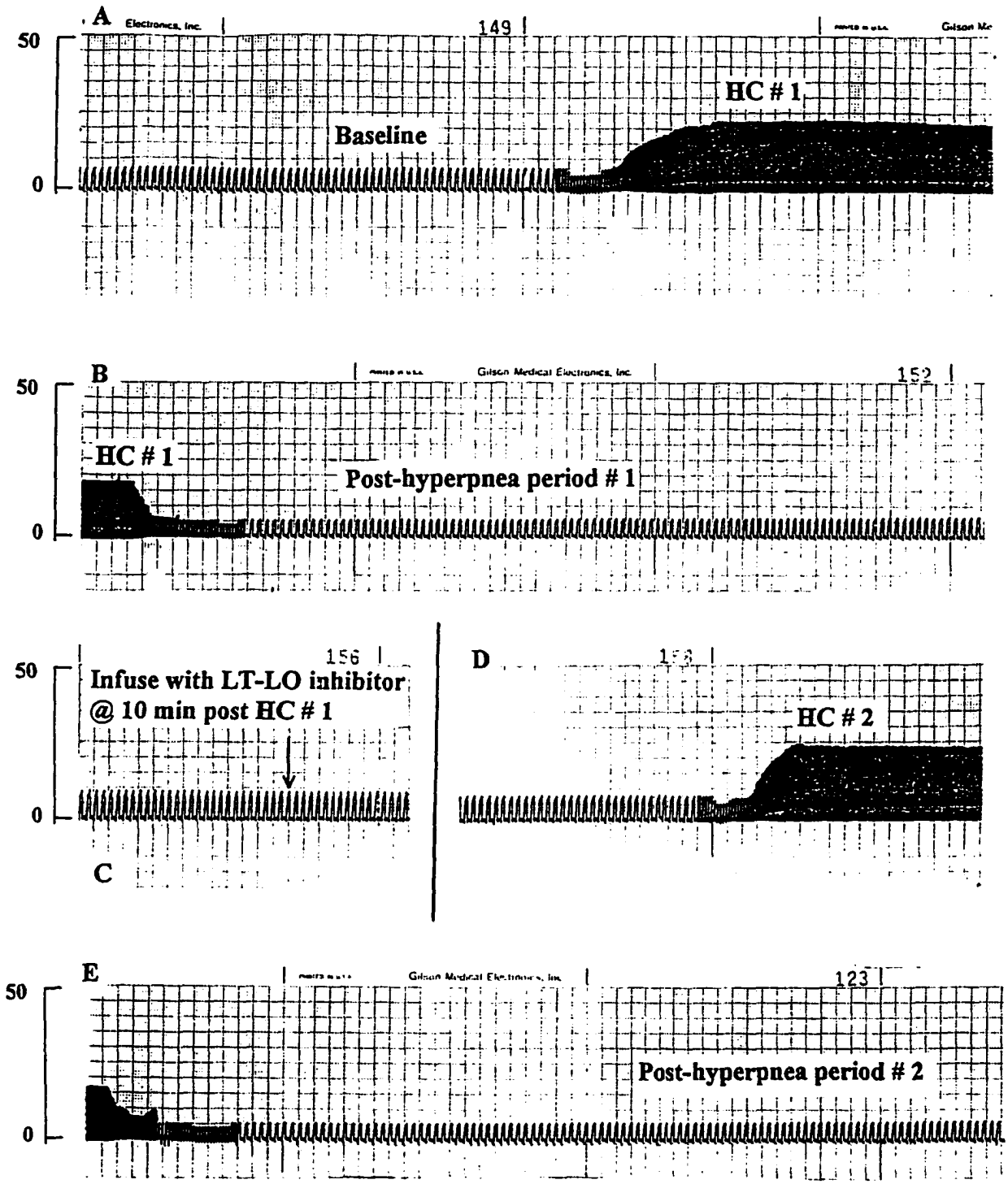


Figure V-10. Example of airway inspiratory pressures for an NSD-BLO animal.

A, Baseline and hyperpnea challenge # 1 (HC # 1). B, HC # 1 and post-hyperpnea period # 1. C, Infusion of LT-LO inhibitor at 10 min post-HC # 1. D, End of post-hyperpnea period # 1 and HC # 2. E, Post-hyperpnea period # 2 . It is evident that HIAO is enhanced during the first 10 min of post-hyperpnea period # 1, and attenuated during post-hyperpnea period # 2, after infusion with the LT-LO inhibitor.

CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

The purpose of the present set of studies was to determine: 1) the influence of dietary salt (NaCl) consumption on post-exercise pulmonary function in subjects with exercise-induced asthma (EIA); 2) which ionic component of NaCl (sodium or chloride) is primarly responsible for the observed changes in post-exercise pulmonary function; and 3) determine whether altering dietary NaCl consumption in guinea pigs (an animal model of EIA) moderates airway responses to dry gas hyperpnea challenge, and to determine whether blocking leukotriene (LT) biosynthesis and the lipoxygenase (LO) pathway mediates the airway response to hyperpnea on the different NaCl diets.

This first study demonstrated that a HSD worsens and a LSD improves post-exercise pulmonary function in EIA subjects, but has no effect on post-exercise pulmonary function in Control (non-asthmatic subjects). The second study showed that it is likely that the chloride constituent of NaCl is largely responsible for decrements in post-exercise flow rates and volumes in EIA subjects. This was accomplished by placing subjects on either a LSD (low sodium, low chloride) or sodium bicarbonate ($NaHCO_3$;

high sodium, low chloride) diet for 2 weeks. Both the LSD and $NaCHO_3$ diet improved post-exercise pulmonary function in EIA subjects compared to the NSD (which had a significantly higher chloride content compared to the other diets). However, the $NaHCO_3$ diet did not improve post-exercise pulmonary function values to the same extent as the LSD. This suggests that the chloride ion may be involved in the effect of dietary NaCl on the severity of EIA. However, it does appear that the sodium ion also plays a role, as high sodium in the $NaHCO_3$ diet prevented the total improvement seen with the LSD. In both studies the LSD did not cure (as compared against Control subjects) but did improve post-exercise pulmonary function in EIA subjects. It is likely that increasing NaCl consumption does not cause EIA, but rather enhances the underlying mechanism.

The third study used guinea pigs as an animal model of EIA, and involved measuring airway responses to dry gas hyperpnea, on either a normal salt (NS) diet or high salt (HS) diet. The HS diet significantly increased airway obstruction compared to the NS diet. In order to delineate the role of LTs in this response, an LT-LO inhibitor was given to each diet group. Blocking both LT biosynthesis and the LO pathway resulted in an attenuation of the bronchoconstrictor response on both diets in response to dry gas hyperpnea. However, the HSD exhibited higher airway resistance compared to the NSD.

The physiological mechanisms by which altering dietary NaCl can mediate the severity of EIA, and NaCl induced - LT release in HIAO are at present unknown. Evidence is beginning to accumulate that asthmatics may be NaCl-sensitive (27). It has long been recognized that a high NaCl intake increases the reactivity of arterioles to

sympathetic stimulation and increases blood pressure in NaCl-sensitive subjects. An increase in the volume of the bronchial microcirculation caused by dietary NaCl loading could exert an important influence on airway diameter. An increase in vascular volume and microvascular pressure, in conjunction with mediator-induced increased vascular permeability, may lead to narrowing of the airways by edema formation. This could compound the effects of increased smooth muscle tone (77) (Figure VI-1). It is now recognized that airway cooling associated with hyperpnea or exercise provokes an increase in the bronchial blood flow in humans and other animals, presumably to regulate thermal losses and prevent tissue injury (7). In asthmatic individuals this effect is manifested as a rapid ressuply of heat (52), which may produce hyperemia and edema at the airway wall by aggravating thermally-induced leakage from a chronically inflamed capillary bed. It has been conclusively shown by direct measurement that the size of the cooling gradient that exists at the end of the hyperpnea determines the intensity of the obstructive response (52). Therefore, the effect of dietary NaCl loading could also augment the effect of airway cooling on the bronchial microcirculation due to exercise in asthmatic subjects

It is possible that high NaCl loads inhibit the Na^+/K^+ ATPase either directly, due to high extracellular sodium, or indirectly by an increase in blood volume causing the release of inhibitors of the ATPase (120). The inhibition would be expected to raise intracellular sodium ($[Na^+]_i$) levels, which subsequently inhibits the Na^+-Ca^{2+} exchanger, resulting in an increase in intracellular calcium ($[Ca^{2+}]_i$) levels, and subsequent smooth muscle contraction (Figure VI-1). This mechanism has been demonstrated in lympthocytes taken from salt-sensitive patients with essential hypertension (2). In

subjects with EIA, it remains to be determined whether NaCl-induced increases in lymphocyte or platelet $[Ca^{2+}]_i$ reflect corresponding increases in bronchial smooth muscle, and whether NaCl-induced increases in $[Na^+]_i$ precede those in $[Ca^{2+}]_i$.

It has been suggested that the chloride ion is responsible for producing a relative metabolic acidosis in NaCl-sensitive subjects, during dietary NaCl loading, resulting in a decrease in intracellular pH ($[pH]_i$). In lymphocytes obtained from a rat model of genetic hypertension, it has been shown that a reduced intracellular $[pH]_i$ stimulates the N^+/H^+ exchange mechanism, promoting an increase in $[Na^+]_i$, and resulting in a rise in $[Ca^{2+}]_i$ via the Na^+/Ca^{2+} exchanger or the Ca^{2+}/H^+ exchanger (12, 13). Although extrapolation of data from cells other than bronchial smooth muscle cells to the pathogenesis of EIA is speculative, it is possible that a reduced $[pH]_i$, and an increase in $[Ca^{2+}]_i$ also occurs in bronchial smooth muscle cells, leading to increased contractility.

It has been shown that mast cells contain Na^+/H^+ antiports, Na^+/K^+ ATPase, and Cl^-/HCO_3 antiports, and that high NaCl loads increase $[Na^+]_i$, which in turn increases $[Ca^{2+}]_i$ via inhibition of the Na^+/Ca^{2+} exchanger, resulting in histamine release. It remains to be determined the effect of dietary NaCl loading on eicosanoid release in mast cells, eosinophils and alveolar macrophages in subjects with EIA.

One interpretation of the third study suggests that high NaCl loads may enhance the release of LTs from effector cells in the airways of guinea pigs subjected to dry gas hyperpnea. An alternative explanation is that NaCl loading may work upstream at the initiating stimulus of EIA and HIAO by altering airway osmolarity, resulting in the subsequent release of inflammatory mediators. Inflammatory mediators may cause bronchial smooth muscle to contract either by direct or indirect (e.g., triggering neural

responses) actions (114) and cause an increase in vascular permeability and airway mucus secretion, which subsequently cause airway obstruction (Figure VI-1). The effect of NaCl-induced release of LTs in airways of EIA subjects has not been determined.

At present, it is a matter of opinion as to whether the results from the first two (human) studies have clinical relevance. The LSD in both studies lessened the severity of EIA, but did not 'cure' symptoms (in comparison to the Control subjects). However, it is interesting to note that in the first study the FEV_1 at 15 minutes post-exercise for EIA subjects following the LSD was reduced by $14 \pm 6\%$ compared to pre-exercise (baseline) values, and in the second study by only $8 \pm 3\%$ (below the 10% threshold that is used to diagnose EIA). Clearly, a clinical study is warranted to determine the importance and magnitude of these dietary changes in post-exercise pulmonary function in exercising asthmatic subjects.

In addressing the specific aims and hypotheses set forth in this study, the following conclusions are drawn:

Study 1:

1. In EIA subjects, tidal volume (V_T) and breathing frequency (f_B) selection during exercise varied between diets, with higher V_T and lower f_B on the HSD, and the opposite on the LSD. This suggests that a HSD causes increased airway resistance compared to the LSD. No changes in V_T and f_B were observed between diets for Control subjects.
2. The LSD improved and the HSD worsened post-exercise pulmonary function in EIA subjects. Compared to pre-exercise values, post-exercise forced expiratory volume in one second (FEV_1) at 15 minutes decreased $14 \pm 6\%$ on the LSD, $20 \pm 7\%$ on the

NSD and decreased further by 24 ± 6% on the HSD, indicating increased airway resistance on the HSD compared to the NSD and LSD. There were no differences observed between diets for post-exercise FEV_1 compared to baseline values for Control subjects.

Study 2:

1. The pattern of exercise ventilation (V_T and f_B) did not differ with diet in EIA subjects. The HSD in the first study and the $NaHCO_3$ (high sodium, low chloride) diet in this study contained the same equimolar concentration of sodium. This suggests that the chloride ion may also be involved in the pulmonary response during exercise in EIA subjects. No differences were observed for the pattern of ventilation in Control subjects.
2. The $NaHCO_3$ diet significantly improved post-exercise pulmonary function, but not to the extent of the LSD, compared to the NSD in EIA subjects. Compared to pre-exercise values, post-exercise FEV_1 at 15 minutes decreased 8 ± 3% on the LSD, 14 ± 3% on the $NaHCO_3$ diet, and 20 ± 3% on the NSD. This suggest less airway resistance on the LSD and $NaHCO_3$ diets compared to the NSD (which had a significantly higher chloride content, as indicated by 24-hour urine excretion of chloride, compared to the LSD and $NaHCO_3$ diet). This implicates the chloride ion in the bronchoconstrictor response to exercise in EIA subjects. No changes were observed between diets on post-exercise pulmonary function in Control subjects.

Study 3:

1. The HS diet (2% NaCl) significantly increased airway obstruction compared to the NS diet (0.75% NaCl) in guinea pigs, after being challenged with dry gas hyperpnea.

2. To examine the mechanism by which a HSD increases post-hyperpnea airway obstruction, guinea pigs, on both diets, were either infused with Nordihydrouaiaretic acid (NDGA), which is non-selective LT biosynthesis and LO pathway inhibitor (BLO group) or saline (CON-group), prior to hyperpnea. The LT-LO inhibitor resulted in a significant reduction in the bronchoconstrictor response to hyperpnea compared to non-blocked groups on the same diets. However, the HSD exhibited higher airway resistance compared to the NSD in the blocked groups. This suggests that NaCl loading may enhance the release of LTs from effector cells in the airways in a dose-dependent manner. An alternative interpretation is that NaCl loading increases airway osmolarity and the subsequent release of inflammatory mediators

Recommendations for Future Research

Human Studies:

1. The time course for the improvement in post-exercise pulmonary function in clinically diagnosed EIA subjects should be measured at every two days, during the two weeks of dietary NaCl restriction.
2. Compare post-exercise pulmonary function values at the end of two weeks on either a HSD, NSD, or potassium bicarbonate ($KHCO_3$) diet in EIA and Control subjects, in order to determine the magnitude of the sodium ion effect on the severity of EIA.
3. Repeat Study 1, and include a methacholine challenge test (at rest) on a subsequent day, while subjects are on the different NaCl diets. The FEV_1 and all indices of airway resistance will be measured after inhalation of normal saline (neutral control

agent), followed by doubling doses of methacholine starting at 0.048 μmol and continuing until FEV_1 decreases to 80% of the post-saline value or a cumulative dose of 12.24 μmol has been administered. A positive test is defined as > 20% decline in FEV_1 (PD_{20}). Airway responsiveness ($\log_{10} PD_{20}$) should be regressed against 24 hour urinary excretion of sodium and potassium, in order to determine if a high rate of sodium excretion is associated with increased reactivity of the airway.

4. Repeat Study 1, and obtain approximately 10 ml of venous blood from each subject using heparinized tubes pre- and post-exercise. The objective will be to determine if manipulation of dietary NaCl alters transmembrane sodium transport systems in erythrocytes and/or leukocytes in EIA subjects compared to Control subjects. Measurements should include: leukocyte sodium influx, estimation of intracellular sodium content, maximal rate (V_{max}) of erythrocyte Na^+/K^+ ATPase activity, Na^+-K^+-Cl^- co-transport, rate constant of sodium leak, along with separate measurements of intracellular calcium concentration. *Note: Extrapolating cell membrane transport systems from leukocytes and erythrocytes to bronchial smooth muscle cells may be misleading, as cellular ion transport mechanisms may differ.*
5. The above mentioned experiment (# 2) should be repeated in clinically diagnosed children with EIA, since EIA is more prevalent in children than adults, and 40-90% of the asthmatic population experience bronchoconstriction upon an exercise challenge. A larger sample size than the one used in the first two studies should be used for the EIA and Control groups. Pharmacological drug use and amount of exercise during the dietary protocol should also be monitored. In addition,

pulmonary function tests should be conducted using a body plethysmograph to measure airway resistance and lung volumes directly.

Animal Studies:

6. Repeat the third (animal) study to determine which inflammatory mediators are primarily responsible for hyperpnea-induced airway obstruction (HIAO) in guinea pigs on different salt diets, by using a cyclooxygenase inhibitor which will block the production of prostanoids, specific LT antagonists, and an inhibitor of the LO pathways. Airway resistance should be measured directly by using an isothermal constant-mass whole body plethysmograph. In addition, arterial blood gases (PO_2, PCO_2 and pH) should be monitored.
7. The above mentioned experiment (# 6) should be repeated, but without eicosanoid inhibitors. Bronchoalveolar lavage (BAL) samples should be obtained from the guinea pig airways in order to determine the number of mediator cell types, such as eosinophils, alveolar macrophages, neutrophils, and mast cells present in the airways at the end of the post-hyperpnea period on the different diets. In addition, a radioimmunoassay (RIA) should be conducted to determine the type of mediators present in the airways and blood on the different NaCl diets. There may be a significant correlation between percentage of BAL mediator cell types and mediators assayed.
8. The above mentioned experiment (# 6) should be repeated using the same eicosanoid inhibitors but with a different dietary protocol. The guinea pigs should be fed a LSD (low sodium, low chloride) or a $NaHCO_3$ diet (high sodium, low

chloride). This study may give insight into which ion is primarily responsible for mediator release.

9. The above mentioned experiment (# 6) should be repeated to determine whether tachykinins or LTs are the central mediators in HIAO in guinea pigs on the different NaCl diets. This study could clarify whether NaCl releases tachykinins directly via stimulation of sensory C-neurons in the airways, which subsequently release LTs from mediator cells present in the airway, or whether NaCl induces LT release directly. This could be accomplished by blocking the LO pathway and the release of tachykinins (pretreated with capsaicin) separately. Subsequently, both capsaicin and the LO inhibitor will be given together to compare the airway response to dry gas hyperpnea, on the two diets. Capsaicin, in sufficient doses has been shown to deplete immunoreactive substance P from nerve fibers in the lung, and to diminish subsequent mucosal and bronchial smooth muscle response to stimuli whose physiological effect is thought to be mediated by the release of tachykinins. Carotid arterial blood and BAL fluid should be obtained for latter determination of substance P (a tachykinin) by enzyme immunoassay (EIA), and LTs (LTC_4, LTD_4, and LTE_4) by RIA.
10. The above mentioned experiment (# 6) should be repeated, without the use of eicosanoid inhibitors. A morphometric examination of the lungs should be conducted by fixing the lungs with liquid nitrogen after taking physiologic measurements and measuring morphometric changes (airway diameter and degree of constriction using 4 μm thick slices of lung tissue) in the airways and tissues; thereby correlating changes in lung function with changes in structure. It is

anticipated that the peak airway resistance would occur approximately 10 minutes after cessation of the hyperpnea challenge. The peak Ptr should be carefully monitored, and the moment it begins to decrease the lungs should be removed immediately from the animal and frozen at -70° C.

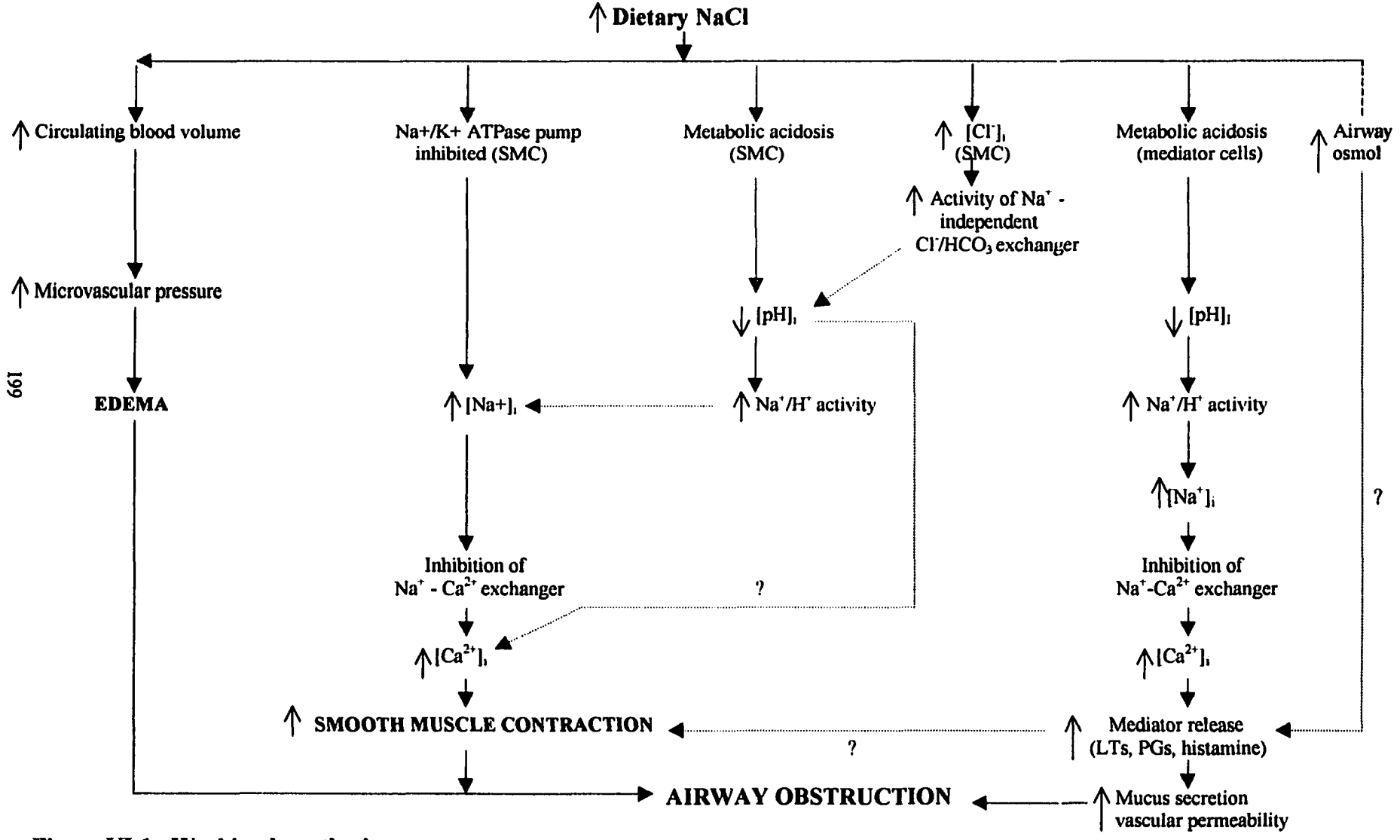


Figure VI-1. Working hypothesis.
Possible interaction of dietary salt loading on circulating blood volume, airway osmolarity, smooth muscle cells and airway mediator cells. This interaction may augment the effects of airway drying and cooling leading to airway obstruction as a result of exercise in EIA subjects

References for Chapters I, II and VI

1. Guidelines for Methacholine and Exercise Challenge Testing-1999. This official statement of the American thoracic society was adopted by the ats board of directors, july 1999. *Am J Respir Crit Care Med* 161: 309-329, 2000.

2. Alexiewicz, J. M., Z. Gaciong, M. Parise, F. Karubian, S. G. Massry, and V. M. Campese. Effect of dietary sodium intake on intracellular calcium in lymphocytes of salt-sensitive hypertensive patients. *Am J Hypertens* 5: 536-541, 1992.

3. Anderson, G. P., H. L. White, and M. R. Fennessy. Increased airways responsiveness to histamine induced by platelet activating factor in the guinea-pig: possible role of lipoxygenase metabolites. *Agents Actions* 24: 1-7, 1988.

4. Anderson, S. D. Exercise-induced asthma. In: *Allergy. Principle and Practice*, edited by E. Middleton, C. E. Reed, E. F. Ellis, N. F. Adkinson and J. W. Yunginger. St. Louis, MO: Mosby-Year Book, Inc, 1993, p. 1343-1367.

5. Anderson, S. D. Is there a unifying hypothesis for exercise-induced asthma? *J Allergy Clin Immunol* 73: 660-5, 1984.

6. Anderson, S. D., R. E. Schoeffel, R. Follet, C. P. Perry, E. Davsikas, and M. Kendall. Sensitivity to heat and water loss at rest and during exercise in asthmatic patients. *Eur J Respir* 63: 459-471, 1982.

7. Baile, E. M., R. W. Dahlby, B. R. Wiggs, G. H. Parsons, and P. D. Pare. Effect of cold and warm dry air hyperventilation on canine airway blood flow. *J Appl Physiol* 62: 526-532, 1987.

8. Barnes, N. C., B. de Jong, and T. Miyamoto. Worldwide clinical experience with the first marketed leukotriene receptor antagonist. *Chest* 111: 52S-60S, 1997.

9. Barnes, P. J. Neurogenic inflammation and asthma. *J Asthma* 29: 165-80, 1992.

10. Barnes, P. J. What is the role of nerves in chronic asthma and symptoms? *Am J Respir Crit Care Med* 153: S5-8, 1996.

11. Barnes, P. J., J. N. Baraniuk, and M. G. Belvisi. Neuropeptides in the respiratory tract. *Am Rev Respir Dis* 144: 1187-1198, 1991.

12. Batlle, D., J. Redon, C. Gutterman, M. LaPointe, A. Saleh, A. Sharma, G. Rombola, M. Ye, W. Alsheikha, L. Gomez, and et al. Acid-base status and intracellular pH regulation in lymphocytes from rats with genetic hypertension. *J Am Soc Nephrol* 5: S12-22, 1994.

13. Batlle, D. C., A. Saleh, and G. Rombola. Reduced intracellular pH in lymphocytes from the spontaneously hypetensive rat. *Hypertension* 15: 97-103, 1990.

14. Beach, J. R., C. L. Young, J. H. Dennis, S. E. Williams, A. J. Avery, and E. H. Walters. A survey of airway responsiveness to methacholine and urinary sodium excretion. *Thorax* 47: 856P, 1992.

15. Beck, K. C., K. P. Offord, and P. D. Scanlon. Bronchoconstriction occurring during exercise in asthmatic subjects. *Am J Respir Crit Care Med* 149: 352-7, 1994.

16. Belcher, N. G., R. Murdoch, N. Dalton, T. J. Clark, P. J. Rees, and T. H. Lee. Circulating concentrations of histamine, neutrophil chemotactic activity, and catecholamines during the refractory period in exercise- induced asthma. *J Allergy Clin Immunol* 81: 100-10, 1988.

17. Belvisi, M. G., C. D. Stretton, M. Miura, G. M. Verleden, S. Tadjkarimi, M. H. Yacoub, and P. J. Barnes. Inhibitory NANC nerves in human tracheal smooth muscle: a quest for the neurotransmitter. *J Appl Physiol* 73: 2505-10, 1992.

18. Ben-Dov, I., I. Gur, E. Bar-Yishay, and S. Godfrey. Refractory period following induced asthma: contributions of exercise and isocapnic hyperventilation. *Thorax* 38: 849-53, 1983.

19. Blackie, S. P., C. Hilliam, R. Village, and P. D. Pare. The time course of bronchoconstriction in asthmatics during and after isocapnic hyperventilation. *Am Rev Respir Dis* 142: 1133-6, 1990.

20. Blosser, S., W. Mitzner, and S. E. Wagner. Effects of increased blood flow on airway morphometry, resistance and reactivity. *J Appl Physiol* 76: 1624-1629, 1994.

21. Boat, T. F., P.-W. Gheng, and M. W. Leigh. Biochemistry of mucus. In: *Airway secretion: physiological basis for the control of mucus hypersecretion*, edited by T. Takishima and S. Shimura. New York: Marcel Dekker, 1994, p. 217-282.

22. Boucher, R. C. Human airway ion transport. *Am J Respir Crit Care Med* 150: 271-281 (Part 1); 581-593 (Part 2), 1994.

23. Broughton, K. S., C. S. Johnson, K. P. Bobin, M. Liebman, and K. M. Kleppinger. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am J Clin Nutr* 65: 1011-1117, 1997.

24. Brown, R. H., E. A. Zerhhouni, and W. Mitzner. Visualization of airway obstruction in vivo during pulmonary vascular engorgement and edema. *J Appl Physiol* 78: 1070-1078, 1995.

25. Burney, P. A diet rich in sodium may potentiate asthma. Epidemiologic evidence for a new hypothesis. *Chest* 91: 143S-148S, 1987.

26. Burney, P. G., J. R. Britton, S. Chinn, A. E. Tattersfield, A. O. Papacosta, M. C. Kelson, F. Anderson, and D. R. Corfield. Descriptive epidemiology of bronchial reactivity in an adult population: results from a community study. *Thorax* 42: 38-44, 1987.

27. Burney, P. G., J. E. Neild, C. H. Twort, S. Chinn, T. D. Jones, W. D. Mitchell, C. Bateman, and I. R. Cameron. Effect of changing dietary sodium on the airway response to histamine. *Thorax* 44: 36-41, 1989.

28. Burney, P. G. J., J. R. Britton, S. Chinn, A. E. Tattersfield, H. S. Platt, A. O. Papacosta, and M. C. Kelson. Response to inhaled histamine and 24 hour sodium excretion. *Br Med J* 292: 1483-86, 1986.

29. Burney, P. G. J., J. R. Britton, S. Chinn, A. E. Tattersfield, H. S. Platt, A. O. Papcosta, and M. C. Kelson. Response to inhaled histamine and 24 hour sodium excretion. *Br Med J* 292: 1483-1486, 1986.

30. Cabral, A. L., G. M. Conceicao, C. H. Fonseca-Guedes, and M. A. Martins. Exercise-induced bronchospasm in children: effects of asthma severity. *Am J Respir Crit Care Med* 159: 1819-23, 1999.

31. Carey, O. J., C. Locke, and J. B. Cookson. Effect of alterations of dietary sodium on the severity of asthma in men. *Thorax* 48: 714-8, 1993.

32. Coles, S. J., K. H. Neill, and L. M. Reid. Effects of leukotrienes C4 and D4 on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 25: 155-170, 1983.

33. Cropp, G. J. The exercise bronchoprovocation test: standardization of procedures and evaluation of response. *J Allergy Clin Immunol* 64: 627-33, 1979.

34. Dahlen, S. E. Pulmonary effects of leukotrienes. *Acta Physiol Scand Suppl* 512: 1-51, 1983.

35. Daviskas, E., I. Gonda, and S. D. Anderson. Local airway heat and water vapour losses. *Respir Physiol* 84: 115-132, 1991.

36. Daviskas, E., I. Gonda, and S. D. Anderson. Mathematical modelling of heat and water transport in human respiratory tract. *J Appl Physiol* 69: 362-372, 1990.

37. de Bisschop, C., H. Guenard, P. Desnot, and J. Vergeret. Reduction of exercise-induced asthma in children by short, repeated warm ups. *Br J Sports Med* 33: 100-4, 1999.

38. Deal, E. C., E. R. McFadden, R. H. Ingram, F. J. Breslin, and J. J. Jaeger. Airway responsiveness to cold air and hyperpnea in normal subjects and in those with hay fever and asthma. *Am Rev Respir Dis* 121: 621-628, 1980.

39. Deal, E. C., E. R. McFadden, R. H. Ingram, and J. J. Jaeger. Hyperpnea and heat flux: initial reaction sequence in exercise-induced asthma. *J Appl Physiol* 46: 476-483, 1979.

40. Dominguez, L. J., M. Barbagallo, G. Di Lorenzo, A. Drago, S. Scola, G. Morici, and C. Caruso. Bronchial reactivity and intracellular magnesium: a possible mechanism for the bronchodilating effects of magnesium in asthma [see comments]. *Clin Sci (Colch)* 95: 137-42, 1998.

41. Drazen, J. M. Leukotrienes as mediators of airway obstruction. *Am J Respir Crit Care Med* 158: S193-200, 1998.

42. Eggleston, P. A. Laboratory evaluation of exercise-induced asthma: methodologic considerations. *J Allergy Clin Immunol* 64: 604-8, 1979.

43. Eggleston, P. A. Methods of exercise challenge. *J Allergy Clin Immunol* 73: 666-9, 1984.

44. Eggleston, P. A., R. R. Rosenthal, S. A. Anderson, R. Anderton, C. W. Bierman, E. R. Bleecker, H. Chai, G. J. Cropp, J. D. Johnson, P. Konig, J. Morse, L. J. Smith, R. J. Summers, and J. J. Trautlein. Guidelines for the methodology of exercise challenge testing of asthmatics. Study Group on Exercise Challenge, Bronchoprovocation Committee, American Academy of Allergy. *J Allergy Clin Immunol* 64: 642-5, 1979.

45. Elliott, P., J. Stamler, R. Nichols, A. R. Dyer, R. Stamler, H. Kesteloot, and M. Marmot. Intersalt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. Intersalt Cooperative Research Group [see comments] [published erratum appears in BMJ 1997 Aug 23;315(7106):458]. *Bmj* 312: 1249-53, 1996.

46. Elmer, P. J., R. H. Grimm, Jr., J. Flack, and B. Laing. Dietary sodium reduction for hypertension prevention and treatment. *Hypertension* 17: I182-9, 1991.

47. Freed, A. N. Models and mechanisms of exercise-induced asthma. *Eur Respir J* 8: 1770-85, 1995.

48. Garrigo, J., I. Danta, and T. Ahmed. Time course of the protective effect of inhaled heparin on exercise-induced asthma. *Am J Respir Crit Care Med* 153: 1702-1707, 1996.

49. Gatto, L. A. pH of mucus in rat trachea. *J Appl Physiol* 50: 1224-1226, 1981.

50. Gelb, A. F., D. P. Tashkin, J. D. Epstein, H. Gong, Jr., and N. Zamel. Exercise-induced bronchodilation in asthma. *Chest* 87: 196-201, 1985.

51. Gilbert, I. A., J. M. Fouke, and E. R. McFadden. The effect of repetitive exercise on airway temperatures. *Am Rev Respir Dis* 142: 826-831, 1990.

52. Gilbert, I. A., and E. R. McFadden. Airway cooling and rewarming. The second reaction sequence in exercise-induced asthma. *J Clin Invest* 90: 699-704, 1992.

53. Haas, F., H. Pineda, K. Axen, D. Gaudino, and A. Haas. Effects of physical fitness on expiratory airflow in exercising asthmatic people. *Med Sci Sports Exerc* 17: 585-92, 1985.

54. Hardy, C. C., C. Robinson, A. E. Tattersfield, and S. T. Holgate. The bronchoconstrictor effect of inhaled prostaglandin D2 in normal and asthmatic men. *N Engl J Med* 311: 209-213, 1984.

55. Hay, D. W. Pharmacology of leukotriene receptor antagonists. More than i nhibitors of bronchoconstriction. *Chest* 111: 35S-45S, 1997.

56. Henrickson, J. M. The effect of inhaled corticosteroids on exercise-induced asthma: a randomized crossover study of budesonide in asthmatic children. *Br Med J* 291: 284-289, 1985.

57. Hoshino, M., and Y. Fukuushima. Effect of OKY-046 (thromboxane A2 synthetase inhibitor) on exercise-induced asthma. *J Asthma* 28: 19-29, 1991.

58. Ichinose, M., H. Inoue, M. Miura, and T. Takishima. Nonadrenergic bronchodilation in normal subjects. *Am Rev Respir Dis* 138: 31-4, 1988.

59. Inman, M. D., R. M. Watson, K. J. Killian, and P. M. O'Byrne. Methacholine airway responsiveness decreases during exercise in asthmatic subjects. *Am Rev Respir Dis* 141: 1414-1417, 1990.

60. Javaid, A., M. J. Cushley, and M. F. Bone. Effect of dietary salt on bronchial reactivity to histamine in asthma. *Br Med J* 297: 454, 1988.

61. Jones, R. S., M. H. Buston, and M. J. Wharton. The effect of exercise on ventilatory function in the child with asthma. *Brit J Dis Chest* 56: 78-86, 1962.

62. Jonsson, E. W. Functional characterisation of receptors for cysteinyl leukotrienes in smooth muscle. *Acta Physiol Scand Suppl* 641: 1-55, 1998.

63. Joris, L., I. Dab, and P. M. Quinton. Elemental composition of human airway surface fluid in health and diseased airways. *Am Rev Respir Dis* 148: 1633-1637, 1993.

64. Kageyama, N., M. Miura, M. Ichinose, M. Tomaki, J. Ishikawa, Y. Ohuchi, N. Endoh, and K. Shirato. Role of endogenous nitric oxide in airway microvascular leakage induced by inflammatory mediators [see comments]. *Eur Respir J* 10: 13-9, 1997.

65. Kemp, J. P., R. J. Dockhorn, and W. W. Busse. Prolonged effect of inhaled salmeterol against exercise-induced bronchospasm. *Am J Respir Crit Care Med* 150: 1612-1615, 1994.

66. Kilburn, K. H. A hypothesis of pulmonary clearance and its implications. *Am Rev Respir Dis* 98: 449-463, 1968.

67. Leff, J. A., W. W. Busse, D. Pearlman, E. A. Bronsky, J. Kemp, L. Hendeles, R. Dockhorn, S. Kundu, J. Zhang, B. C. Seidenberg, and T. F. Reiss. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction [see comments]. *N Engl J Med* 339: 147-52, 1998.

68. Lieberman, D., and D. Heimer. Effect of dietary sodium on the severity of bronchial asthma [see comments]. *Thorax* 47: 360-2, 1992.

69. Lundberg, J. M., K. Alving, J. A. Karlsson, R. Matran, and G. Nilsson. Sensory neuropeptide involvement in animal models of airway irritation and of allergen-evoked asthma. *Am Rev Respir Dis* 143: 1429-30; discussion 1430-1, 1991.

70. Mahler, D. A. Exercise-induced asthma. *Med Sci Sports Exerc* 25: 554-61, 1993.

71. Makker, H. K., and S. T. Holgate. Mechanisms of exercise-induced asthma. *Eur J Clin Invest* 24: 571-85, 1994.

72. Manning, P. J., R. M. Watson, D. J. Margolskee, V. C. Williams, J. I. Schwartz, and P. M. O'Byrne. Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D4-receptor antagonist [see comments]. *N Engl J Med* 323: 1736-9, 1990.

73. Manning, P. J., R. M. Watson, and P. M. O'Byrne. Exercise-induced refractoriness in asthmatic subjects involves leukotriene and prostaglandin interdependent mechanisms. *Am Rev Respir Dis* 148: 950-4, 1993.

74. McFadden, E. R. Exercise-induced asthma. A historical review and modern concepts. In: *Exercise-Induced Asthma*, edited by E. R. McFadden. New York: Marcel Dekker, Inc, 1999, p. 1-10.

75. McFadden, E. R., Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon [see comments]. *Lancet* 335: 880-3, 1990.

76. McFadden, E. R., Jr., and I. A. Gilbert. Exercise-induced asthma. *N Engl J Med* 330: 1362-7, 1994.

77. McFadden, E. R., and I. A. Gilbert. Exercise-induced asthma as a vascular phenomenon. In: *Exercise-Induced Asthma*, edited by E. R. McFadden. New York: Marcel Dekker, Inc, 1999, p. 115-135.

78. McFadden, E. R., K. A. M. Lenner, and K. P. Strohl. Postexertional airway rewarming and thermally induced asthma. *J Clin Invest* 78: 18-25, 1986.

79. McFadden, E. R., B. M. Pichurko, and H. F. Bowman. Thermal mapping of the airways in humans. *J Appl Physiol* 58: 564-570, 1985.

80. McGiff, J. C., and J. R. Vane. Prostaglandins and the regulation of blood pressure. *Kidney Int Suppl*: S262-70, 1975.

81. Mickleborough, T. D., L. Cordain, R. W. Gotshall, and A. Tucker. A low sodium diet improves indices of pulmonary function in exercise-induced asthma. *J Exerc Physiol* 3: 46-54, 2000.

82. Mohsenin, V., A. B. Dubois, and J. S. Douglas. Effects of ascorbic acid on response to methacholine challenge in asthmatic subjects. *Am Rev Respir Dis* 127: 143-147, 1983.

83. Ninan, T., and G. Russel. Respiratory symptoms and atopy in Aberdeen school children, evidence for two surveys 25 years apart. *Brit Med J* 304: 873-875, 1992.

84. Novembre, E., G. Frongia, E. Lombardi, M. Resti, E. Zammarchi, and A. Vierucci. Respiratory pathophysiologic responses: the protective effect and duration of action of two doses of inhaled furosemide on exercise-induced asthma in children. *J Allergy Clin Immunol* 96: 906-909, 1995.

85. O'Byrne, P. M. Exercise-induced bronchoconstriction: elucidating the roles of leukotrienes and prostaglandins. *Pharmacotherapy* 17: 31S-38S, 1997.

86. O'Byrne, P. M. Leukotrienes in the pathogenesis of asthma. *Chest* 111: 27S-34S, 1997.

87. O'Byrne, P. M., and G. L. Jones. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 134: 69-72, 1986.

88. Okayama, H., T. Aikawa, M. Okayama, H. Sasakin, S. Mue, and T. Takishima. Brochodilating effects of intravenous magnesium sulfate in bronchial asthma. *JAMA* 257: 1076-1078, 1987.

89. Omori, C., B. H. Schofield, W. Mitzner, and A. N. Freed. Hyperpnea with dry air causes time-dependent alterations in mucosal morphology and bronchovascular permeability. *J Appl Physiol* 78: 1043-1051, 1995.

90. O'Sullivan, S. On the role of PGD2 metabolites as markers of mast cell activation in asthma. *Acta Physiol Scand Suppl* 644: 1-74, 1999.

91. Parm, J., C. E. Horton, J.-N. Nencia-Huerta, F. House, N. M. Eiser, J. T. H. Clark, B. W. Spur, and T. N. Lee. Effect of dietary supplementation with fish oils on lipds on mild asthma. , 1988.

92. Pistelli, R., F. Forastiere, G. M. Corbo, V. Dell'Orco, G. Brancato, N. Agabiti, A. Pizzabiocca, and C. A. Perucci. Respiratory symptoms and bronchial responsiveness are related to dietary salt intake and urinary potassium excretion in male children. *Eur Respir J* 6: 517-22, 1993.

93. Randolph, C., M. Randolph, and B. Fraser. Exercise-induced asthma in school children. *J Allergy Clin Immunol* 87: 341, 1991.

94. Rundell, K. W., R. L. Wilber, L. Szmedra, D. M. Jenkinson, L. B. Mayers, and J. Im. Exercise-induced asthma screening of elite athletes: field versus laboratory exercise challenge [In Process Citation]. *Med Sci Sports Exerc* 32: 309-16, 2000.

95. Rupp, N. T., M. F. Guill, and D. S. Brudno. Unrecognized exercise-induced bronchospasm in adolescent athletes. *Am J Dis Child* 146: 941-944, 1992.

96. Saria, A., C.-R. Martling, Z. Yan, E. Theordorsen-Norheim, R. Gamse, and J. M. Lundberg. Release of multiple tachykinins from capsaicin-sensitive nerves in the lung bradykinin, histamine, dimethylphenyl, piperazinium and vagal nerve stimulation. *Am Rev Respir Dis* 137: 1330-1335, 1988.

97. Serafina, S. M., and E. D. Michaelson. Length and distribution of cilia in human and canine airways. *Bul Eur Physiopathol Respir* 13: 551-559, 1977.

98. Shimizu, T., H. Mochizuki, M. Shigeta, and A. Morikawa. Effect of inhaled indomethacin on exercise-induced bronchoconstriction in children with asthma [see comments]. *Am J Respir Crit Care Med* 155: 170-3, 1997.

99. Smith, L. Childhood asthma: diagnosis and treatment. *Curr Probl Prediatr* 23: 271-305, 1993.

100. Souhrada, J. F., and M. Souhrada. Provocative challenge by exercise or hyperventilation. In: *Provocation testing in clinical practice*, edited by S. L. Spector. New York: Marcel Dekker, 1995, p. 425-449.

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115. Waite, D. A., E. F. Eyles, S. L. Tonkin, and T. V. O'Donnell. Asthma prevalence in Tokelauan children in two environments. *Clin Allergy* 10: 71-75, 1980.

116. Walters, E. H. Dual effect of prostaglandin E2 on normal airways smooth muscle in vivo. *Thorax* 37: 918-922, 1982.

117. Walters, E. H., P. M. O'Byrne, L. M. Fabbi, P. D. Graf, M. J. Holtzman, and J. A. Nadel. Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. *J Appl Physiol* 57: 129-134, 1984.

118. Wanner, A. Clinical aspects of mucociliary transport. *Am Rev Respir Dis* 116: 73-125, 1977.

119. Weiler, J. M. Exercise-induced asthma: a practical guide to definitions, diagnosis, prevalence, and treatment. *Allergy Asthma Proc* 17: 315-25, 1996.

120. Weissberg, P. L., M. J. West, M. J. Kendall, M. Ingram, and K. L. Woods. Effect of changes in dietary sodium and potassium on blood pressure and cellular electrolyte handling in young normotensive subjects. *J Hypertens* 3: 475-80, 1985.

121. Welsh, M. J. Electrolyte transport by airway epithelia. *Physiol Rev* 67: 1143-1184, 1987.

122. Woods, J. W., J. F. Evans, D. Ethier, S. Scott, L. Vickers, L. Hearn, J. A. Heibein, S. Charleson, and I. I. Singer. 5-lipoxygenase-activating and 5-lipoxygenase activating protien are localized in the nuclear envelope of activated human leukocytes. *J Exp Med* 178: 1935-1946, 1990.

APPENDIX A

REGRESSION ANALYSIS

FIRST AND SECOND STUDY

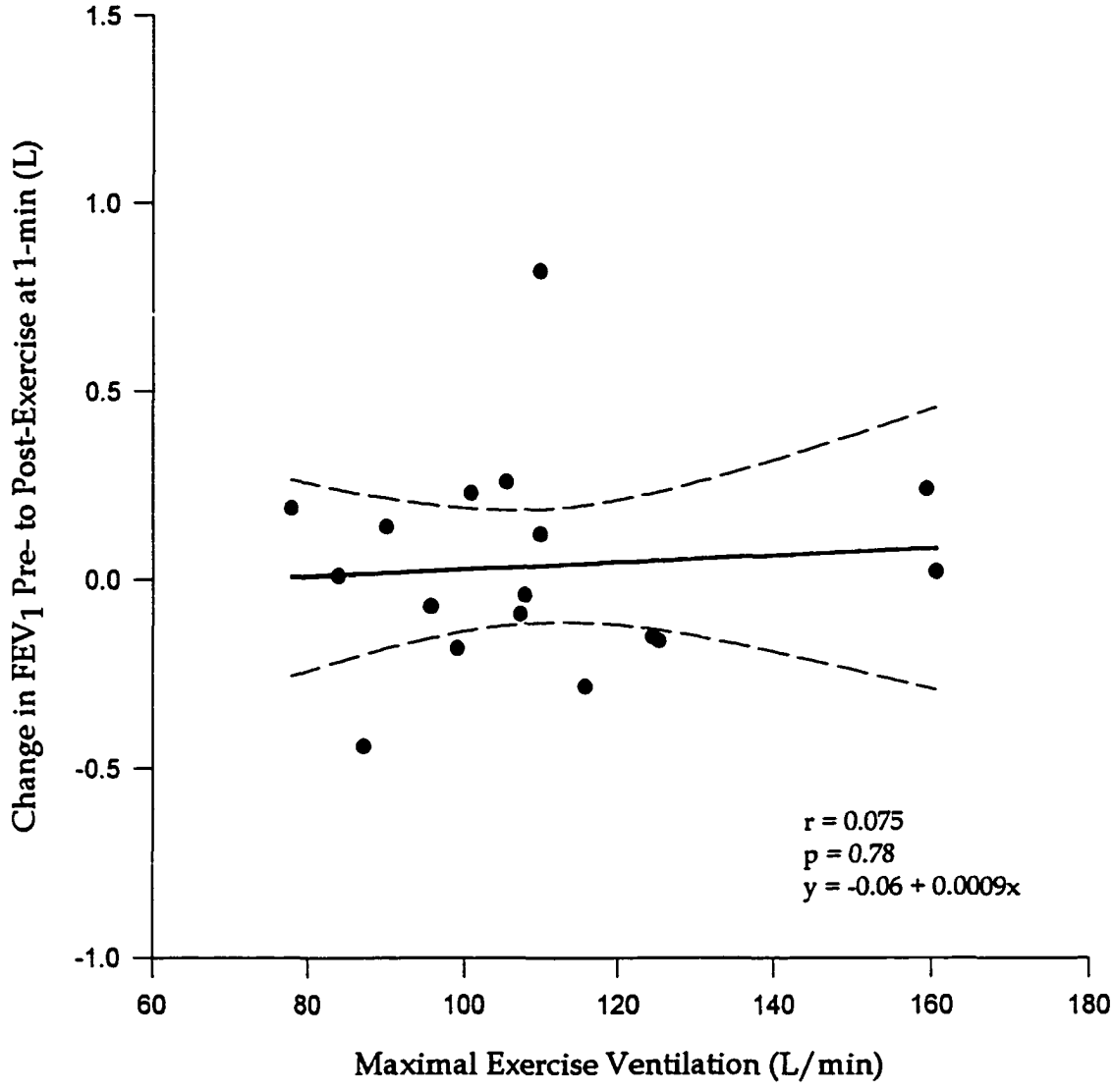


Figure A-1. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 1-minute of recovery in Control subjects.

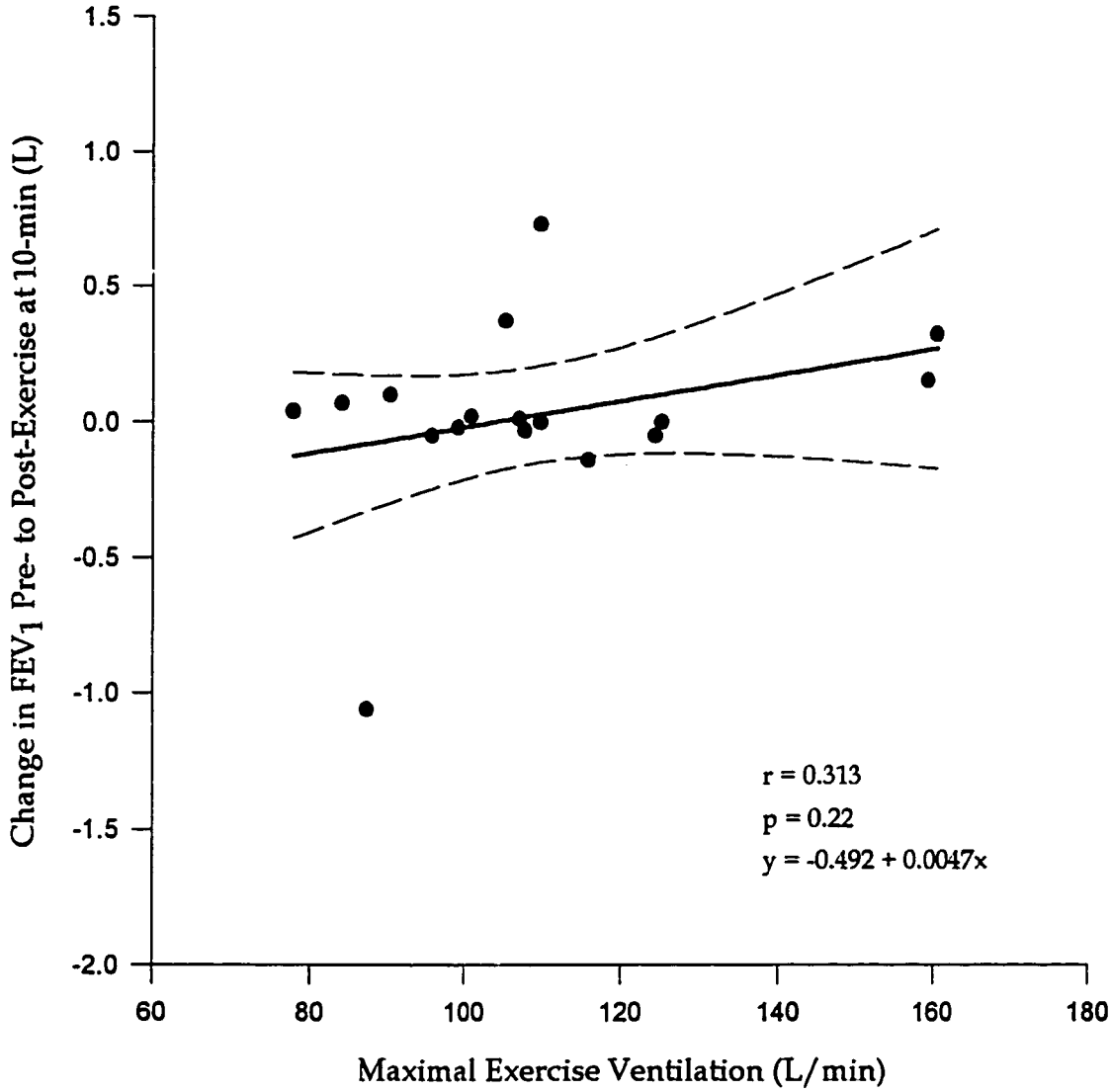


Figure A-2. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 10-minutes of recovery in Control subjects.

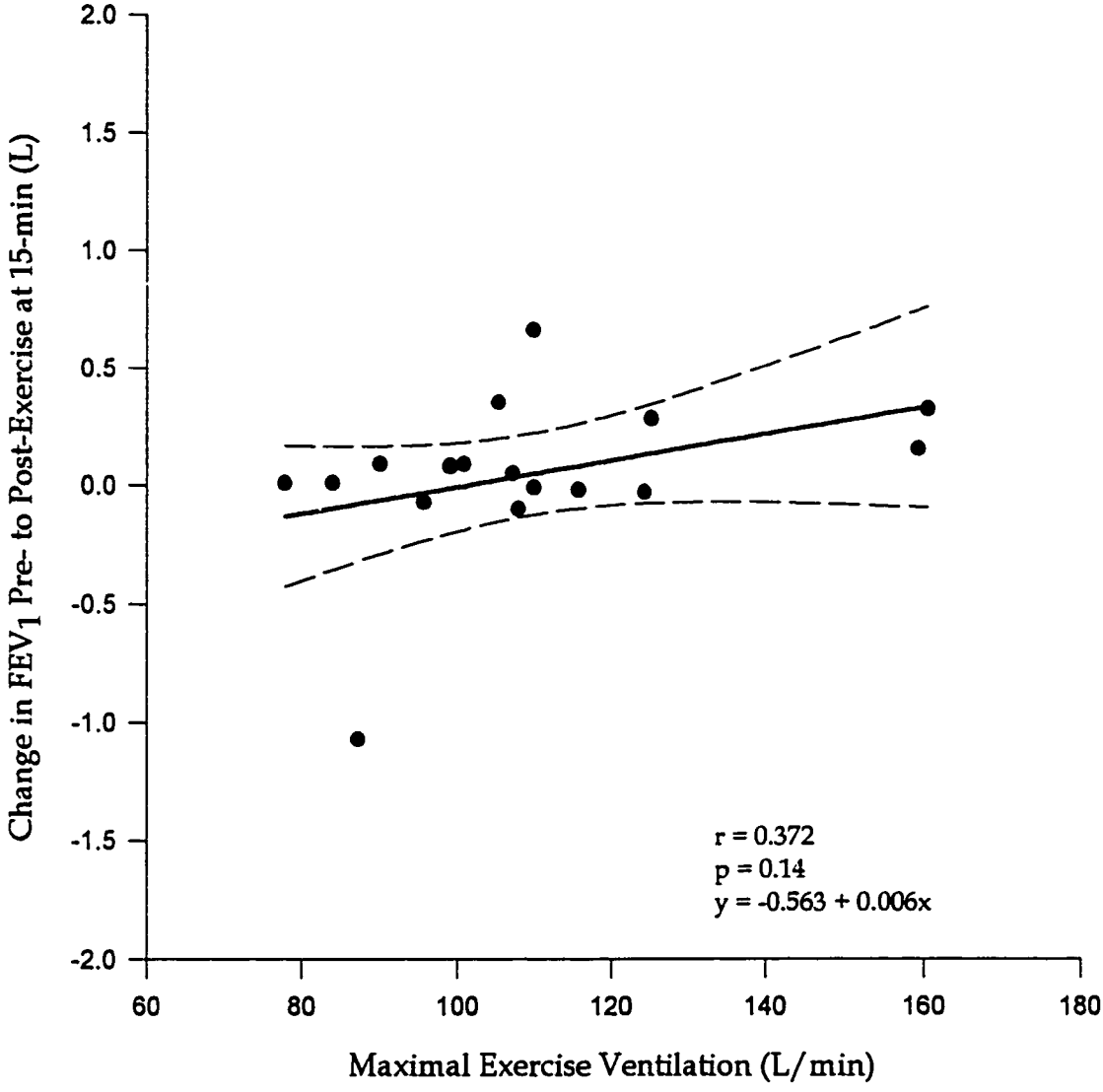


Figure A-3. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 15-minutes of recovery in Control subjects.

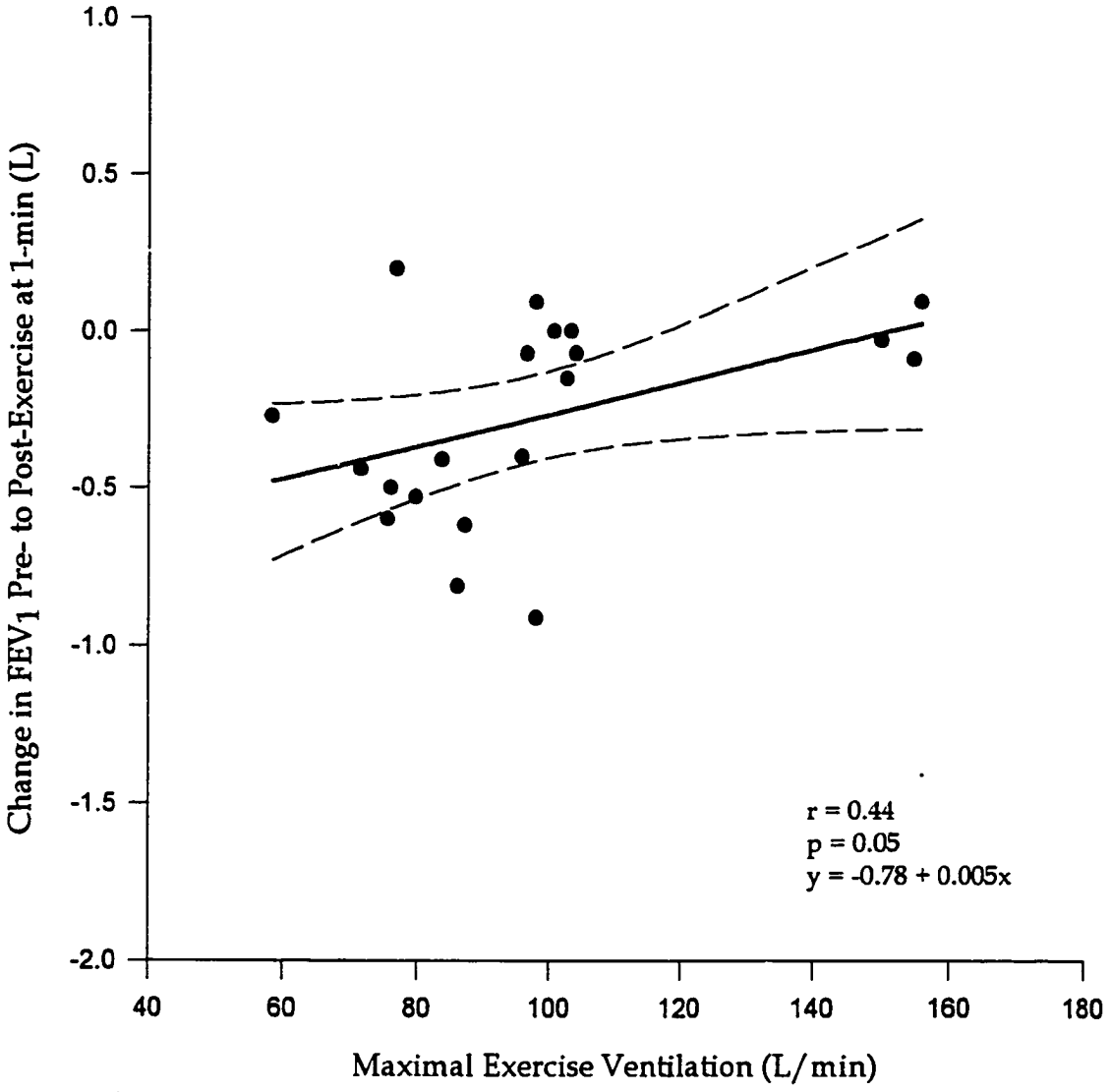


Figure A-4. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 1-minute of recovery in EIA subjects.

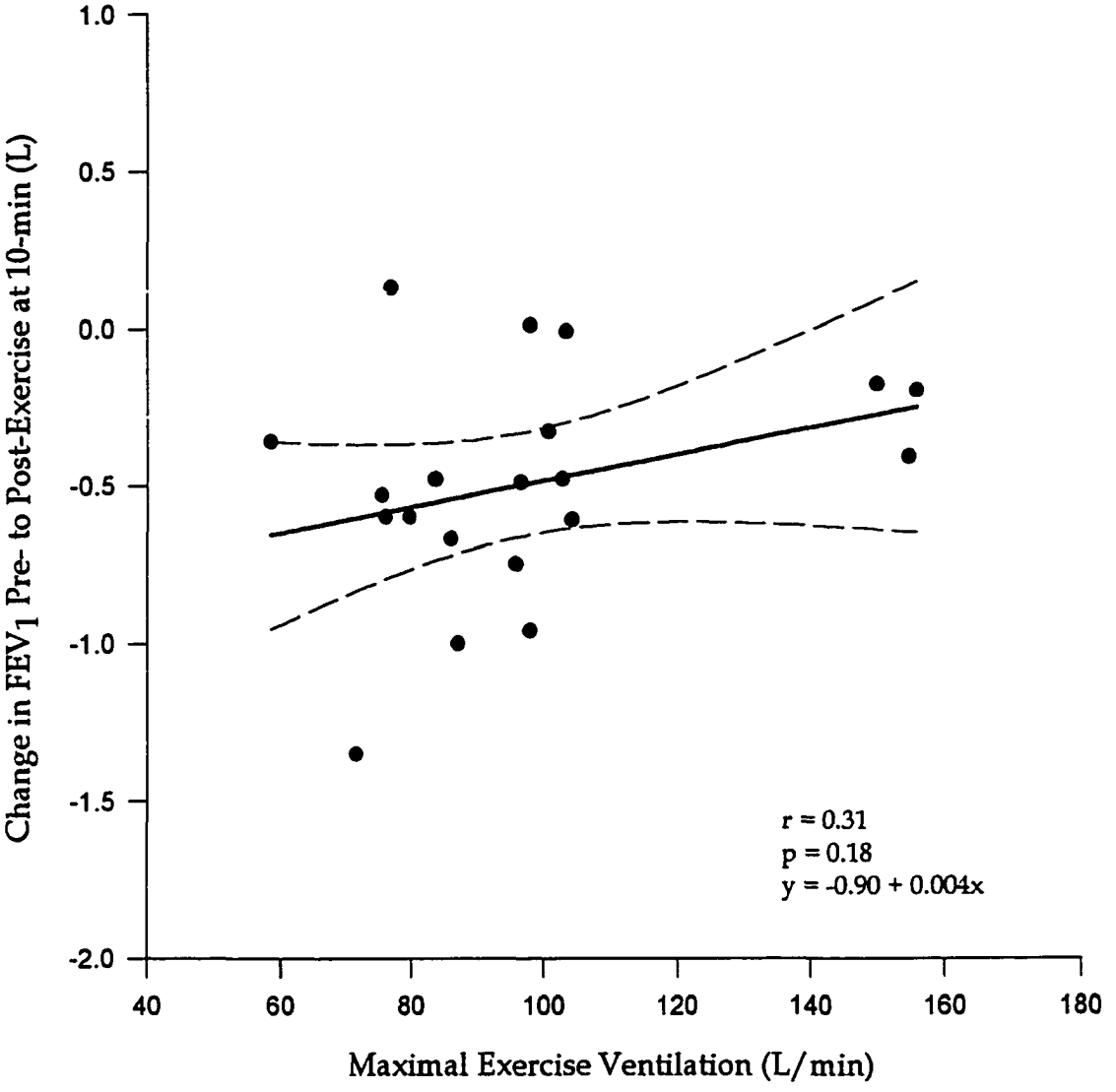


Figure A-5. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 10-minutes of recovery in EIA subjects.

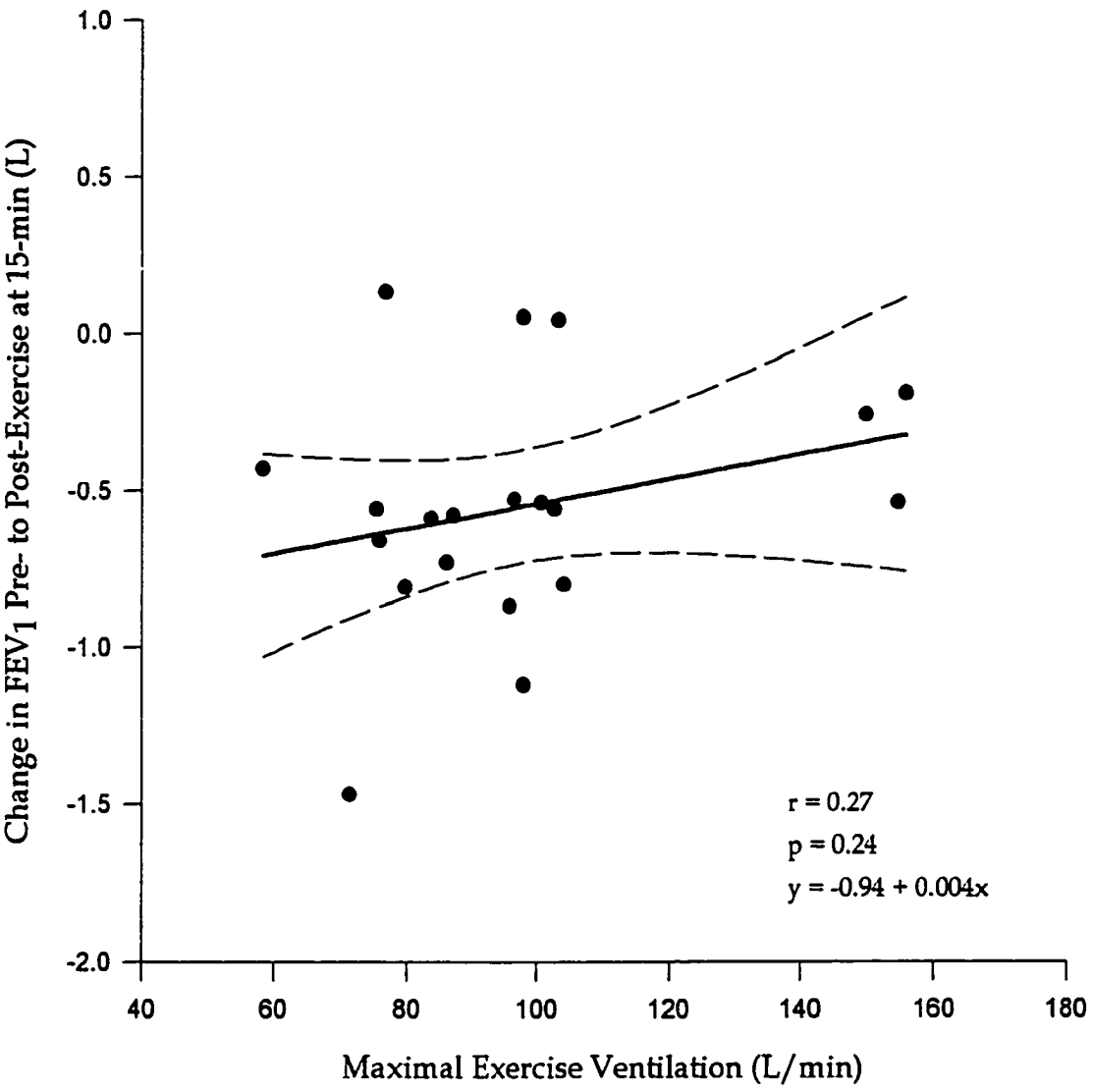


Figure A-6. First study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 15-minutes of recovery in EIA subjects.

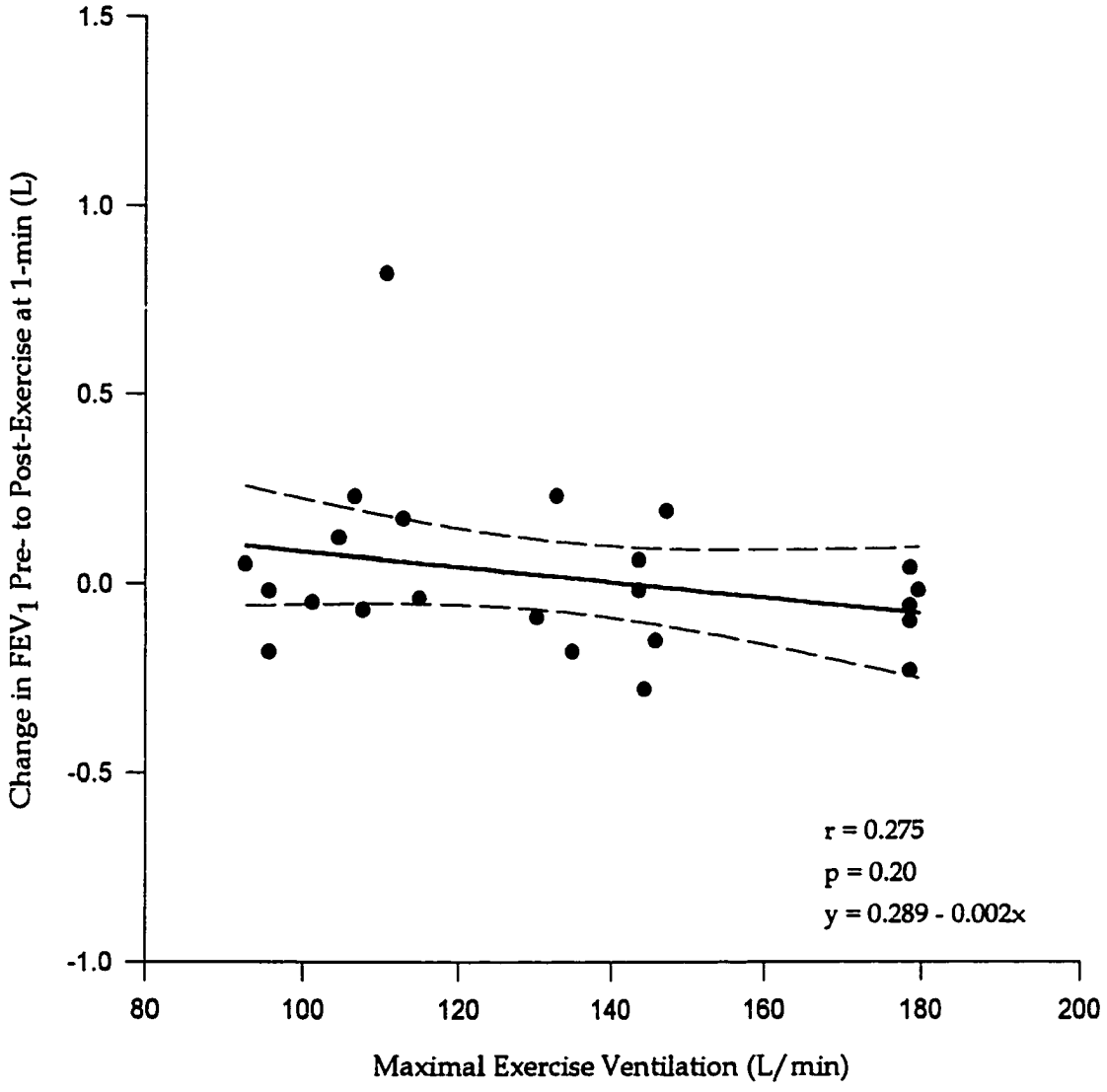


Figure A-7. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 1-min of recovery in Control subjects.

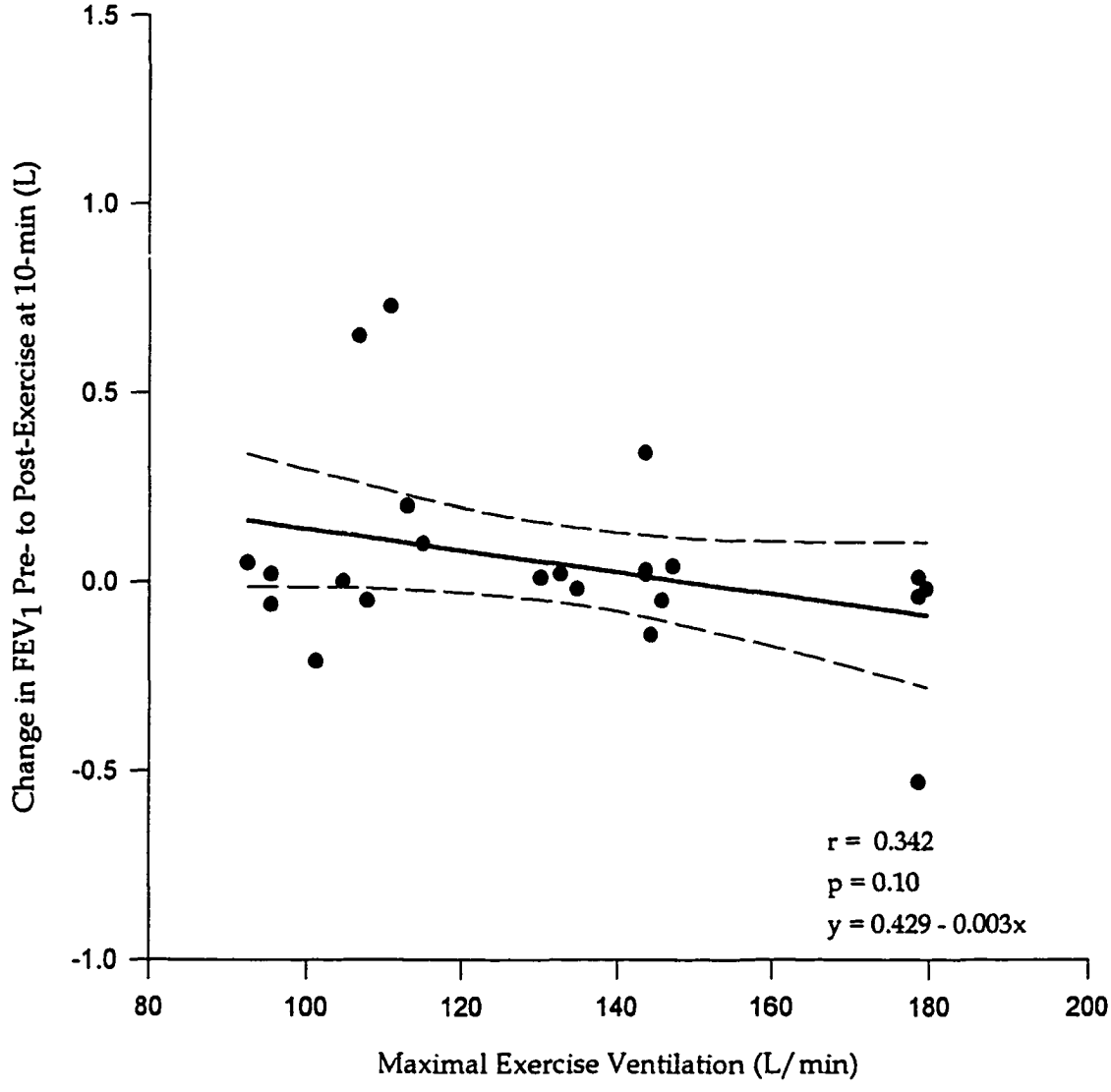


Figure A-8. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 10-min of recovery in Control subjects.

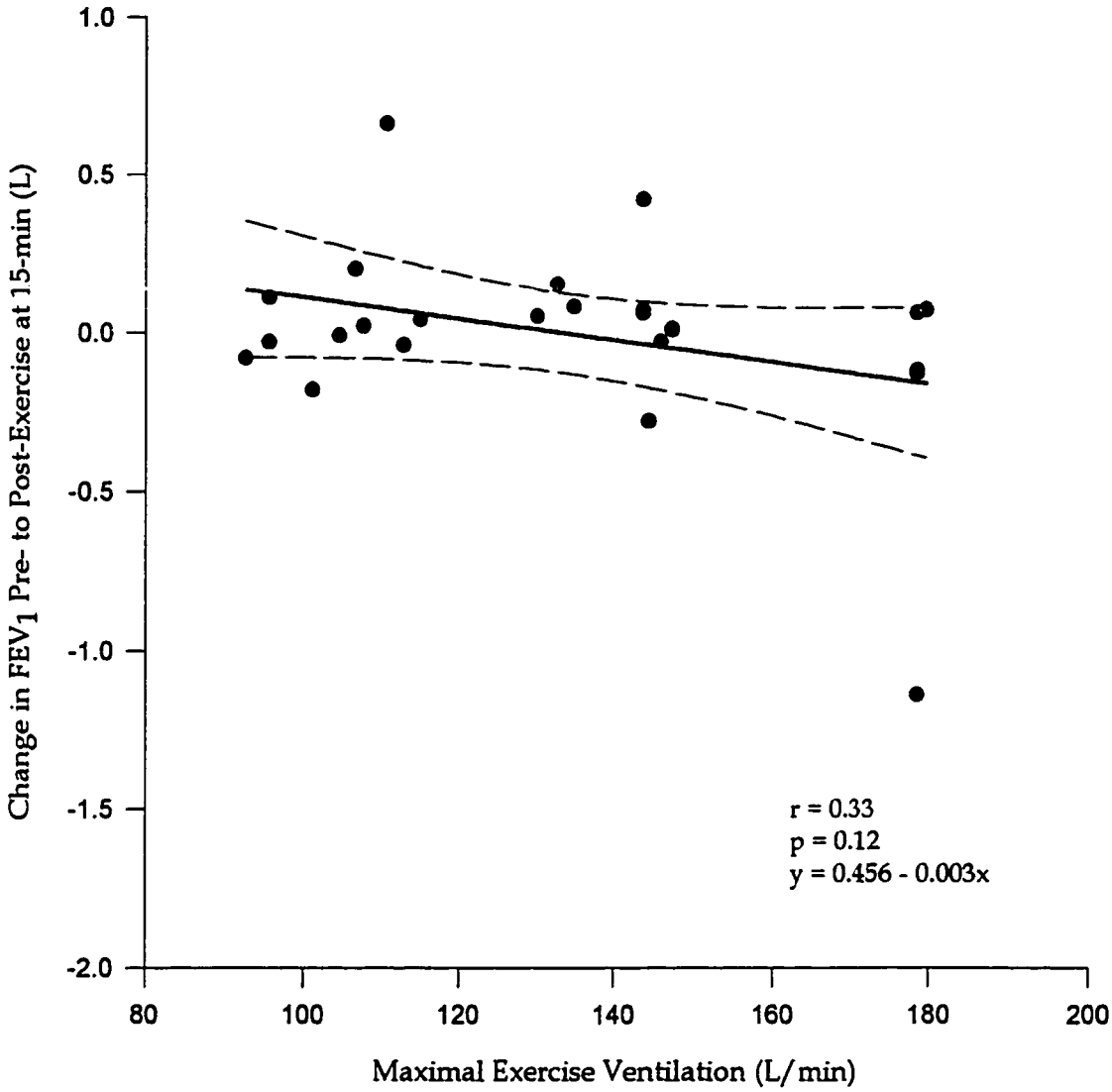


Figure A-9. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 15-min of recovery in Control Subjects.

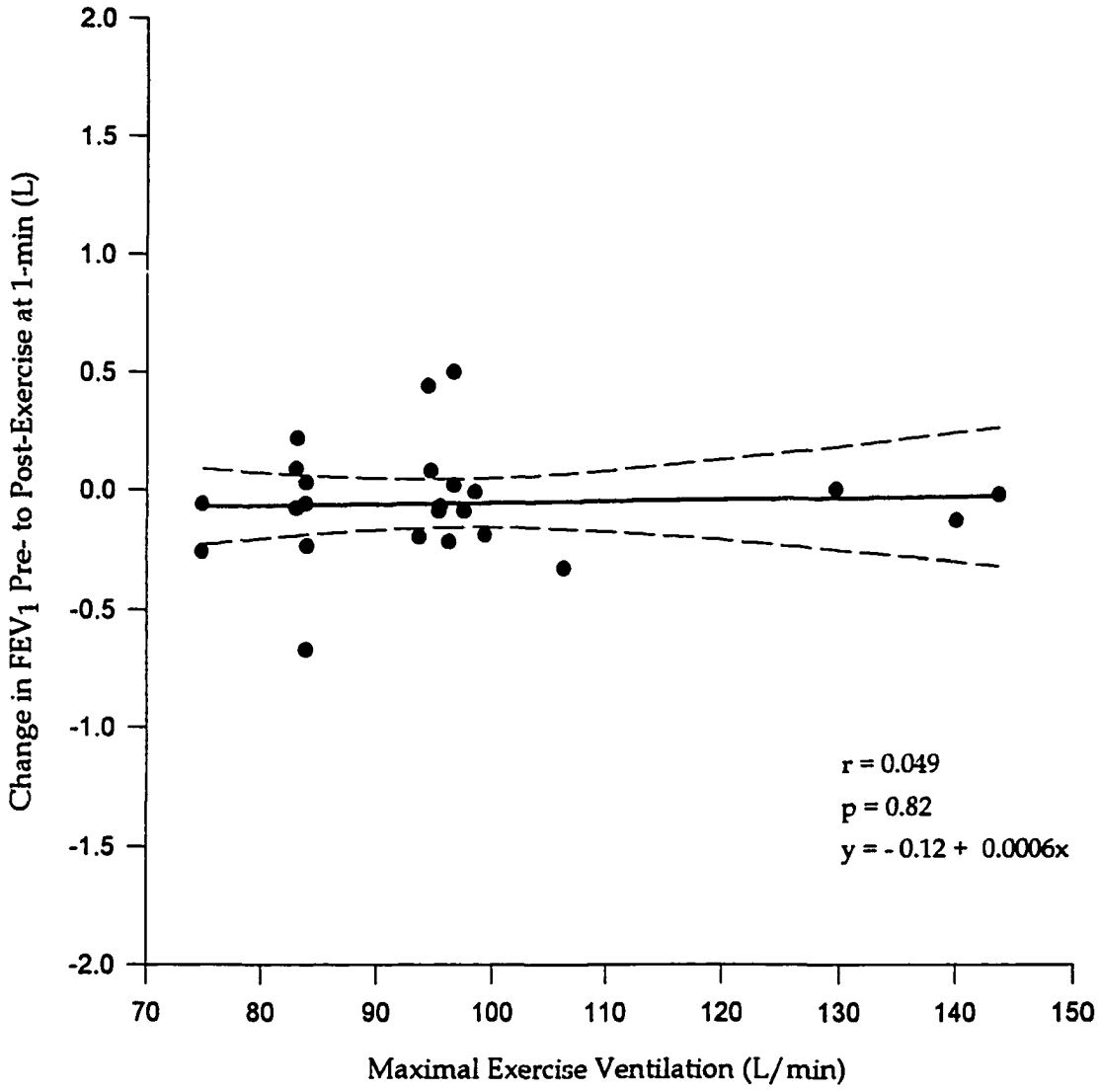


Figure A-10. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 1-min of recovery in EIA subjects.

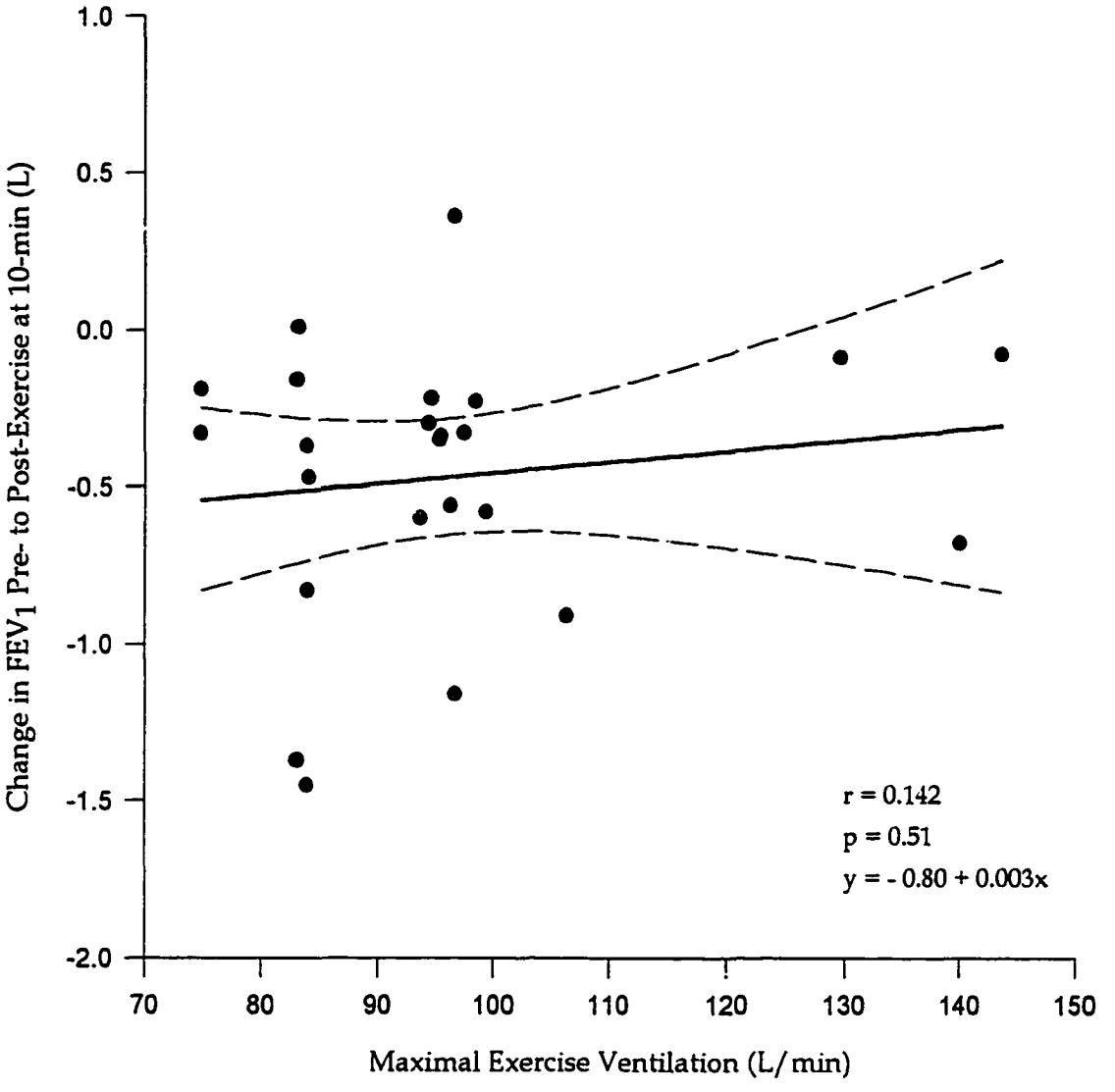


Figure A-11. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 10-min of recovery in EIA subjects.

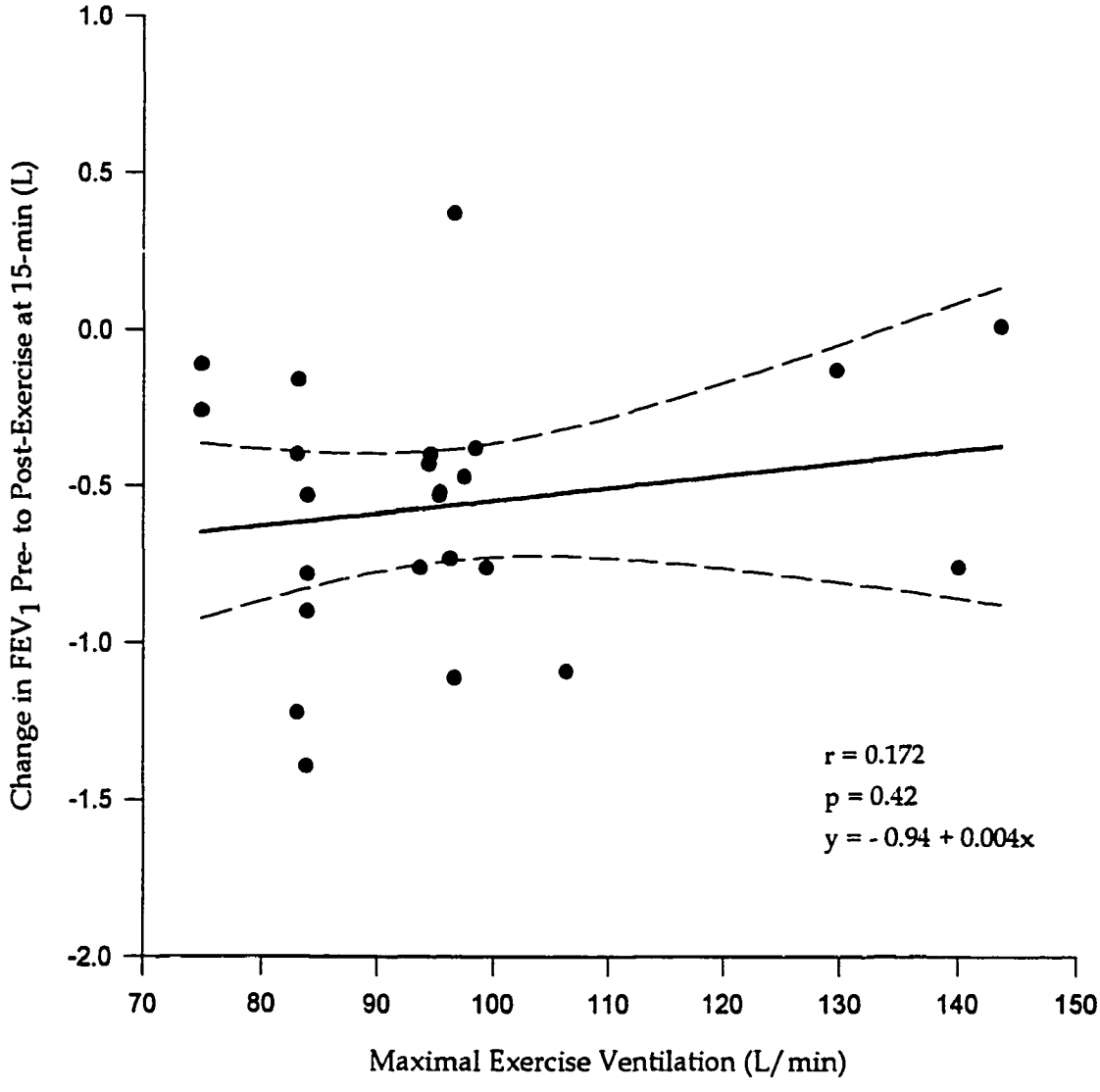
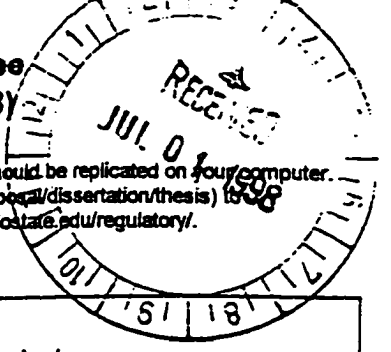


Figure A-12. Second study. Regression of maximal exercise ventilation and pre- to post-exercise changes in FEV_1 at 15-min of recovery in EIA subjects.

APPENDIX B

HUMAN AND ANIMAL RESEARCH PROJECT APPROVAL FORMS

Colorado State University Human Research Committee
Application to Use Human Subjects (H-100, Rev 4/98)



Complete Part A and Part B. On separate pages, list all questions from Part C and respond to each as applicable. Part C should be replicated on your computer. For full review protocols, return the ORIGINAL (with original signatures) and 11 copies (each with all attachments except proposal/dissertation/thesis) to: Regulatory Compliance, 410 University Services Center. Assistance is available on our web page at http://www.research.colostate.edu/regulatory/.

Action of the CSU Human Research Committee

- ☐ Approved as **EXEMPT** research Approval number: _____ Period: _____
- ☒ Approved as **NON-EXEMPT** research Approval number: 98-158H Period 8/4/98-8/4/99
  - ☒ Conditions: use of approved consent form
  - ☐ Other (*see attachment for details*)
- ☐ Cannot be approved as currently submitted (*see attached memo for details*)
- ☐ Tabled (*see attached memo for details*)

HRC Authorization: Celia S Wah Date: 8/4/98

Part A. COVER SHEET ☒ New Protocol ☐ Resubmission

1. Project Title: Dietary Sodium Intake Mediates Cellular Sodium Transport and Bronchial Reactivity in Asthmatics.

2. Principal Investigator (PI): Robert Gotshall, Ph.D. **3. Telephone:** 491-6374

4. Department: Exercise and Sport Science **5. E-mail:** gotshall@cahs.colostate.edu

6. Co-Principal Investigator: C.W. Miller, Ph.D.; Tim Mickleborough **7. Telephone:** 491-7842

8. Department: Physiology **9. E-mail:** cwmiller@cvmbs.colostate.edu

10. If Co-PI is a student, is this project for a: ☐ Thesis ☒ Dissertation Pilot Project ☐ Neither
(Attach thesis/dissertation prospectus, abstract, or methodology chapter.)

11. Date project activity to begin: Upon approval by HRC

12. Will this project be supported by external funds? ☐ Yes (answer 13-15) ☒ No (go to signatures)

13. Funding Agency (attach proposal or methodology section):

14. Grant/contract number: **15. Proposal deadline:**

As the PI submitting this proposed research and signing below, I agree to conduct the research involving human subjects as presented in the protocol or modifications to it and as approved by the Department and the Human Research Committee; to obtain and document informed consent and provide a copy of the consent form to each subject unless this is waived by the HRC; to present any proposed modifications in the research to the HRC for review and approval prior to implementation; to retain records for the mandated lengths of time; and to report to the HRC any problems or injuries to subjects.

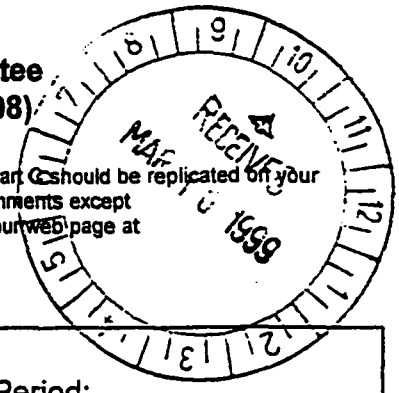
PI Signature: Robert Gotshall Date: 6/26/98

Department Chair/Head or Acting (circle which) Signature

My signature below confirms that I have read this protocol and approve of this research.

Signature: Richard J. Sforza Jr. Date: 6/26/98

Colorado State University Human Research Committee
Application to Use Human Subjects (H-100, Rev 4/98)



Complete Part A and Part B. On separate pages, list all questions from Part C and respond to each as applicable. Part C should be replicated on your computer. For full review protocols, return the ORIGINAL (with original signatures) and 11 copies (each with all attachments except proposal/dissertation/thesis) to Regulatory Compliance, 410 University Services Center. Assistance is available on our web page at http://www.research.colostate.edu/regulatory/.

Action of the CSU Human Research Committee

- ☐ Approved as **EXEMPT** research Approval number: _____ Period: _____
- ☒ Approved as **NON-EXEMPT** research Approval number: 99-059H Period: 4/14/99 - 4/14/00
  - ☒ Conditions: use of approved consent form
  - ☐ Other (*see attachment for details*)
- ☐ Cannot be approved as currently submitted (*see attached memo for details*)
- ☐ Tabled (*see attached memo for details*)

HRC Authorization: Celia B Wade Date: 5.19.99

Part A. COVER SHEET ☒ New Protocol ☐ Resubmission

1. **Project Title:** Dietary Chloride Intake on Severity of Exercise-Induced Asthma.
2. **Principal Investigator (PI):** Robert Gotshall, Ph.D. **3. Telephone:** 491-6374
4. **Department:** Exercise and Sport Science **5. E-mail:** gotshall@cahs.colostate.edu
6. **Co-Principal Investigator:** C.W. Miller, Ph.D.; Tim Mickleborough **7. Telephone:** 491-7842
8. **Department:** Physiology **9. E-mail:** cwmiller@cvmbs.colostate.edu
10. **If Co-PI is a student, is this project for a:** ☐ Thesis ☒ Dissertation ☐ Neither Pilot Project
    (Attach thesis/dissertation prospectus, abstract, or methodology chapter.)
11. **Date project activity to begin:** Upon approval by HRC
12. **Will this project be supported by external funds?** ☐ Yes (answer 13-15) ☒ No (go to signatures)
13. **Funding Agency** (attach proposal or methodology section):
14. **Grant/contract number:** **5. Proposal deadline:**

As the PI submitting this proposed research and signing below, I agree to conduct the research involving human subjects as presented in the protocol or modifications to it and as approved by the Department and the Human Research Committee; to obtain and document informed consent and provide a copy of the consent form to each subject unless this is waived by the HRC; to present any proposed modifications in the research to the HRC for review and approval prior to implementation; to retain records for the mandated lengths of time; and to report to the HRC any problems or injuries to subjects.

PI Signature: Robert Gotshall Date: 3/5/99

Department Chair/Head or Acting (circle which) Signature

My signature below confirms that I have read this protocol and approve of this research.

Signature: Richard G. Lund Date: 3/5/99

Colorado State University Animal Care and Use Committee
Animal Research/Teaching Protocol Approval

Principal Investigator: Miller,Charles W Phone: 970-491-7842
Co-Investigator(s):

Department: Physiology
College: Vet. Medicine and Biomedical Sciences

Protocol Number: 99-191A-01
This number is required to place animal orders with Laboratory Animal Resources.

Project Title: Role of Dietary Salt on Airway Reactivity During Hyperpnea and Histamine Exposure in Guinea Pigs
Approval Date: 17-AUG-99 Approval Expiration Date: 17-AUG-00

Species Approved: 30 adult male guinea pigs/yr

If the number of animals ordered exceeds the number approved by 10%, a justification for more animals must be sent to the Regulatory Compliance Coordinator before further orders will be processed by the Laboratory Animal Resources. Use form A-103.

Funding Agency: n/a

This project was reviewed by the Animal Care and Use Committee and action taken as follows:

___ Project approved without condition

_X_ Project approved with the following conditions:

One of the LAR veterinarians must observe the first procedure.

Chair: Ed Hoover LLC

Date: 8/17/99

Questions concerning this approval should be directed to Linda L. Kovar, Coordinator, Animal Care and Use Committee, 410 University Services Center (1-0232, lkovar@research.colostate.edu)

APPENDIX C

SUBJECT INFORMED CONSENT

Colorado State University
Informed Consent to Participate in a Research Project

Title of Project: Dietary Sodium Intake Mediates Cellular Sodium Transport and Bronchial Reactivity in Asthmatics.

Name of Principal Investigator: Robert Gotshall, Ph.D.

Name of Co-Investigators: C.W. Miller, Ph.D.; Tim Mickleborough, M.S.

Contact Name and Phone Number: Robert Gotshall, Ph.D., 491-6374

Purpose of the Research: The purpose of this research study is to determine if increased salt in the diet will worsen symptoms of asthma, especially during exercise, and whether a diet low in salt will reduce asthmatic symptoms associated with exercise. You are being asked to volunteer about nine (9) hours of your time over a five (5) week period.

Procedures/Methods to be Used: The general procedures for which you are volunteering are as follows. There will be an initial screening test day in which you will be explained the details of the study and will undergo an exercise test, with your lung function tested before and after the exercise. This will take about one (1) hour. You will then be scheduled to return to the laboratory for the first testing day of the study, this will take about 2 hours of your time. Prior to coming to the laboratory, you will collect your urine for a 24-hour period in containers provided to you. This is so we can check how much salt you have eaten, because it passes through your body. You will also be asked not to exercise within the 12 hours prior to coming to the laboratory, and not to use your medication (if you use any) for 24 hours prior to coming to the laboratory (this will be discussed with you each time, since different medications may require a different procedure). Should you require the use of your medication, let us know and we will reschedule your test.

Upon arrival at the laboratory, the following will occur on each visit. You will have a small (10 ml) blood sample drawn from a vein in your arm. You will then have electrodes placed on your chest to monitor your heart function and cuffs placed on your arms to measure blood pressure. You will then have your heart function measured by echocardiography, which requires placing a small, hand-held, device against your chest. Your blood vessels in your neck and arm will also be examined in a similar manner to determine how your blood vessels function. Following this testing, you will proceed to the exercise lab, where you will blow into a tube as hard as you can to determine your lung function. You will then exercise on a treadmill for about 10 to 15 minutes as the workload is slowly increased until you can no longer continue (maximal exercise test). During the exercise, you will have your heart monitored by the electrocardiogram (ECG), blood pressure taken by a cuff on your arm, and breathe into a tube to permit the measurement of how much oxygen you are consuming. You will also have the amount of oxygen in your blood continuously monitored by a small device which connects to your ear. Following the exercise, you will again have your lung function measured at 1, 5, 10 and 15 minutes after the exercise. You may stop the test at any time if you become too uncomfortable. Each testing day will take about 2 hours of your time.

There will be a total of four (4) testing days, after your initial screening test. Following your first full testing day, you will be given a special menu which you must follow when eating so we can control the amount of salt which you eat. You will also be given some capsules to take each day, which may or may not contain salt. You will remain on this diet for two (2) weeks. At the end of the two weeks, you will again save you urine for 24 hours, and refrain from exercise and medications as you did previously. You will then come to the laboratory as scheduled and the tests run again. You will then return to your normal diet for a week, collect your urine, refrain from exercise and medications and be tested again, as before. Following this, you will again start the special diet plus capsules to take for an additional two weeks and repeat all procedures.

Five Week Period:

Initial Screening Test	→ First Test Day	→ Second Test Day	→ Third Test Day	→ Fourth Test Day
	Normal Diet	Special Diet	Normal Diet	Special Diet
		2 weeks	1 week	2 weeks

Risks Inherent in the Procedures: Maximal exercise includes the risk of fatigue, muscle strains, heart abnormalities (arrhythmias), 0.01% chance of death (in cardiac population), 0.02% risk of cardiac arrhythmias requiring hospitalization (in a cardiac population), and change of blood pressure. The ECG will be monitored for abnormalities. In an individual with asthma/EIA, the risks increase somewhat with the possibility of minor chest pain/discomfort, and significant respiratory distress. Oxygen saturation will be monitored and the exercise stopped if saturation drops by 10 points. If respiratory distress occurs, the exercise will be stopped and you will be permitted use of your medication. Oxygen by mask is available if your blood oxygen is slow to recover. You may stop the testing at any point. Should you require medical assistance, 911 emergency access is available in the laboratory.

There are very few risks associated with acute exposure to a high salt diet within normal subjects. Some possible risks are a transient elevation in blood pressure. Certain subjects may have an increased sensitivity to a high salt diet and show a large rise in blood pressure and subsequently an increase in sodium and water retention (hypertensive response). Thus, blood pressure will be taken on the first day of the study and then every first and third day of the treatment period. If diastolic blood pressure rises greater than 5 mmHg compared to baseline values, you will be returned to a lower salt diet and removed from the study.

There are no known risks involved with a low salt intake or the vascular testing. There is a risk of bruising, infection, local soreness, and fainting with the venipuncture. If you are taking any nonprescribed (or illegal) drugs, such as cocaine, your risks will be increased and you should not participate in this study. Please be aware that it is impossible to identify all potential risks in an experimental procedure, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

Benefits: You will receive your maximal exercise data, which can be useful to indicate your fitness and can be used to guide training. The asthmatic population may benefit, if the low salt diet reduces symptoms of asthma.

Confidentiality: You will not be identified on any data or in any publication of results of this study. All records will be kept secured in the principal investigator's files.

Liability: The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions about subjects' rights may be directed to Celia Walker at (970) 491-1563.

Participation: Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 2 pages.

_____
Participant name (printed)

_____ _____
Participant signature Date

_____ _____
Investigator signature Date

Colorado State University
Informed Consent to Participate in a Research Project

Title of Project: Dietary Chloride Intake on Severity of Exercise-Induced Asthma.

Name of Principal Investigator: Robert Gotshall, Ph.D.

Name of Co-Investigators: C.W. Miller, Ph.D.; Tim Mickleborough, M.S.

Contact Name and Phone Number: Robert Gotshall, Ph.D., 491-6374

Purpose of the Research: The purpose of this research study is to determine if dietary intake of salt (sodium chloride) influences the severity of exercise-induced asthma. Specifically, this study address the question of whether it is sodium or chloride which is most important in this regard.

Procedures/Methods to be Used: The general procedures for which you are volunteering are as follows. There will be an initial screening test day in which you will be explained the details of the study and will undergo an exercise test, with your lung function tested before and after the exercise. This will take about one (1) hour. You will then be scheduled to return to the laboratory for the first testing day of the study, this will take about 2 hours of your time. Prior to coming to the laboratory, you will collect your urine for a 24-hour period in containers provided to you. This is so we can check how much salt you have eaten, because it passes through your body. You will also be asked not to exercise within the 12 hours prior to coming to the laboratory, and not to use your 'rescue' medication for 24 hours prior to coming to the laboratory (this will be discussed with you each time, since different medications may require a different procedure). Should you require the use of your medication, let us know and we will reschedule your test.

Upon arrival at the laboratory, the following will occur on each visit. You will report to the exercise lab, where you will blow into a tube as hard as you can to determine your lung function. You will then exercise on a treadmill for about 10 to 15 minutes as the workload is slowly increased until you can no longer continue (maximal exercise test). During the exercise, you will have your heart monitored by the electrocardiogram (ECG), blood pressure taken by a cuff on your arm, and breathe into a tube to permit the measurement of how much oxygen you are consuming. You will also have the amount of oxygen in your blood continuously monitored by a small device which connects to your ear. Following the exercise, you will again have your lung function measured at 1, 5, 10 and 15 minutes after the exercise. You may stop the test at any time if you become too uncomfortable. Each testing day will take about 2 hours of your time.

There will be a total of four (4) testing days, after your initial screening test. Following your first full testing day, you will be given a special menu which you must follow when eating so we can control the amount of salt (sodium chloride, table salt) which you eat. You will also be given some capsules to take each day, which may or may not contain a special salt (sodium bicarbonate). You will remain on this diet for two (2) weeks. At the end of the two weeks, you will again save you urine for 24 hours, and refrain from exercise and medications as you did previously. You will then come to the laboratory as scheduled and the tests run again. You will then return to your normal diet for a week, collect your urine, refrain from exercise and medications and be tested again, as before. Following this, you will again start the special diet plus capsules to take for an additional two weeks and repeat all procedures.

Five Week Period:

Initial Screening Test	→ First Test Day	→ Second Test Day	→ Third Test Day	→ Fourth Test Day
	Normal Diet	Special Diet	Normal Diet	Special Diet
		2 weeks	1 week	2 weeks

Risks Inherent in the Procedures: Maximal exercise includes the risk of fatigue, muscle strains, heart abnormalities (arrhythmias), 0.01% chance of death (in cardiac population), 0.02% risk of cardiac arrhythmias requiring hospitalization (in a cardiac population), and change of blood pressure. The ECG will be monitored for abnormalities. In an individual with asthma/EIA, the risks increase somewhat with the possibility of minor chest pain/discomfort, and significant respiratory distress. If your pulmonary function is below normal prior to exercise, you will not be exercised. Oxygen saturation will be monitored and the exercise stopped if saturation drops by 10 points. If respiratory distress occurs, the exercise will be stopped and you will be permitted use of your medication. Oxygen by mask is available if your blood oxygen is slow to recover. You may stop the testing at any point. Should you require medical assistance, 911 emergency access is available in the laboratory.

There are very few risks associated with acute exposure to a high sodium diet within normal subjects. Some possible risks are a transient elevation in blood pressure. Certain subjects may have an increased sensitivity to a high sodium diet and show a large rise in blood pressure and subsequently an increase in sodium and water retention (hypertensive response). Thus, blood pressure will be taken on the first day of the study and then every first and third day of the treatment period. If diastolic blood pressure rises greater than 5 mmHg compared to baseline values, you will be returned to a lower salt diet and removed from the study.

The special salt, sodium bicarbonate, may cause stomach or intestinal upset. The dose of this salt is split so that you do not take the full dose all at once to reduce this possibility.

There are no known risks involved with a low salt intake. If you are taking any nonprescribed (or illegal) drugs, such as cocaine, your risks will be increased and you should not participate in this study. Please be aware that it is impossible to identify all potential risks in an experimental procedure, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

Benefits: You will receive your maximal exercise data, which can be useful to indicate your fitness and can be used to guide training. The asthmatic population may benefit, if the low salt diet reduces symptoms of asthma.

Confidentiality: You will not be identified on any data or in any publication of results of this study. All records will be kept secured in the principal investigator's files.

Liability: The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions about subjects' rights may be directed to Celia Walker at (970) 491-1563.

Participation: Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 2 pages.

_____
Participant name (printed)

_____ _____
Participant signature Date

_____ _____
Investigator signature Date

APPENDIX D

GUIDELINES FOR SODIUM RESTRICTION

GUIDELINES FOR SODIUM CONTROLLED DIET
1500 mg/day (56 mEq/day)

Sodium, a mineral, is abundant in many foods. It may be found naturally, or it may be added during the processing of a food. The most common form of salt is composed of sodium and chloride. Reducing your sodium intake will require changing your eating behavior.

You are being asked to go on a sodium-restricted diet (1500mg/day or 56 mEq/day) for the duration of the study. On this diet, no salt should be added in cooking. In addition, we are asking that you use low-sodium milk and low and no-salt free bread.

Most foods contain some sodium, which means that the salt added by nature is low. Therefore, fresh vegetables, fruits, grains, milk, and meat are not limited by this diet.

Processed foods are generally high in sodium, however, it is possible to buy low- or no-salt processed foods. The following additives contain sodium in high amounts and should be avoided:

- salt (sodium chloride)
- monosodium glutamate (MSG)-present in seasonings
- sodium nitrite and nitrate-used in the curing of meat

The following guidelines will help you reduce the sodium in your diet:

- stop using the salt shaker
- omit salt in cooking and in baking
- substitute sodium-free seasonings and spices
- use a salt substitute (potassium chloride) only with a doctor's permission
- include a variety of fresh, unprocessed foods in your diet, such as fruits, grains and most vegetables
- use low-sodium commercial products
- sugars, oils, shortenings, and unsalted butter and margarine are negligible sources of sodium

Please note that a tablespoon of salt contains 2000 mg of sodium and therefore table salt should be avoided. The most common processed foods that are high in sodium and that should be avoided include, but not limited to processed meat, salsa and chips, packaged noodles, soups (unless low in sodium), frozen dinners, pizza (unless home made), etc.

Whether you are a control (non-asthmatic) or asthmatic subject, there are many health benefits to be gained from lowering your dietary salt consumption. At present, salt intake among adults and children in the United States averages at least 9000mg/day, with large numbers of adults consuming 12000mg/day, resulting in 10-15 times the basal sodium requirement for adults and growing children of 500mg/day of salt.

At the end of each two week dietary period we will collect a 24-hr urine sample from you (containers supplied) which will monitor compliance to the diet. Unfortunately, if you have not kept to the dietary protocol then we can not use your pulmonary function and exercise data in the study.

FOODS ALLOWED

- low-sodium bread, rolls and crackers
- fresh vegetables
- vegetables such as potato, sweet potato it emit salt when cooking
- fresh fruits
- low-sodium pasta sauce and tomato paste
- fruit juices, salt-free vegetable juices
- dry cereals such as puffed rice, puffed wheat, shredded wheat, low-sodium cornflakes
- pasta, noodles, macaroni, and spaghetti cooked without salt
- meat, fish, poultry, eggs and low-sodium cheese
- natural peanut butter
- unsalted butter and margarine
- cream cheese and sour cream
- most soft drinks
- unsalted nuts and pretzels
- cranberry sauce
- syrups
- one to two eggs per day
- coffee, tea

FLAVORING AIDS ALLOWED

- low sodium ketchup
- salt-free herb and blend spices
- spices such as basil, cinnamon, etc
- lemon and lime juice
- dry mustard
- vinegar
- garlic or onion powder
- paprika
- pepper (lemon pepper contains salt and should be avoided)
- tabasco sauce

FOODS TO AVOID

- all canned vegetables, unless low in sodium
- frozen vegetables, unless low in sodium
- commercial foods made with milk (eg., chocolate milk, condensed milk, etc)
- dried fruit with sodium sulfite added
- processed cheese
- yeast breads, rolls, or breads and muffins made from commercial mixes containing baking powder, baking soda, salt or MSG
- salted popcorn, pretzels, waffles
- salted or smoked meat (eg., corned beef, frankfurters, etc)
- salted or smoked fish (eg., anchovies, canned salmon,etc)
- shellfish (eg., shrimp, crab, etc)
- commercial peanut butter (eg., skippy)
- salted butter or margarine
- commercial salad dressings
- instant cocoa mixes
- commercial candies, cakes, cookies, etc

FLAVORINGS TO AVOID

- wocestershire sauce
- BBQ sauce
- catsup
- chili sauce
- garlic or onion salt
- horseradish prepared with salt
- meat sauces, tenderizers, regular steak sauce
- MSG
- prepared mustard
- olives
- pickles and relishes
- soy sauce (low-sodium soy sauce is still "high" in sodium)
- sugar substitutes containing sodium
- regular bouillon cube

APPENDIX E

ANIMAL STUDY INFORMATION

BIO-SERV® A Holton Industries Co.
Products That Perform
One 8th Street, Suite 1, Frenchtown, NJ 08825
Phone: 908-996-2155 • Web Address: www.bio-serv.com • Fax: 908-996-4123

CERTIFICATE OF ANALYSIS

2/24/00

COLORADO STATE UNIVERSITY
DR. TIM MICKLEDOROUGH
LAB ANIMAL RES., PAINTER CTR.
DEPT. OF PHYSIOLOGY
200 W. LAKE STREET
FORT COLLINS CO 80523

SALES ORDER NUMBER:	51472
INVOICE NUMBER:	77712
INVOICE DATE:	2/23/00
CUSTOMER PURCHASE ORDER NUMBER:	459825
DIET PRODUCT NUMBER:	F4097
DIET LOT NUMBER:	41021
DIET DESCRIPTION:	GUINEA PIG DIET, GRAIN-BASED, SALT (2%), 3/16" PELLET

PROXIMATE PROFILE

	THEORETICAL	ACTUAL
PROTEIN	17.20%	19.40%
FAT	3.70%	2.83%
FIBER	11.60%	10.89%
ASH	7.70%	8.99%
MOISTURE <	10.00%	7.76%
CARBOHYDRATE (CALCULATED)	51.80%	50.13%

CALORIC PROFILE

PROTEIN	0.734	0.828	kcal/ GRAM
FAT	0.327	0.250	kcal/ GRAM
CARBOHYDRATE	2.051	1.985	kcal/ GRAM
ETHANOL			kcal/ GRAM
TOTAL	3.112	3.063	kcal/ GRAM

This assay certifies that the above diet is guaranteed to meet the above theoretical parameters as specified by Bio-Serv and/or the investigator.

Anayltical variability, sampling variability, and moisture levels account for overall differences in theoretical and actual figures for assays.

Information contained herein is believed to be correct and reliable. However, Bio-Serv does not assume responsibility for it or for recommendations of our representatives inasmuch as conditions and methods of use are beyond our control. Further, we make no warranty, expressed or implied, of any kind regarding these products or their use, and the purchaser assumes all risks of use or handling either in accordance with directions or not.

Assays performed by: Independent Analytical Laboratory
Method of Reference: AOAC-10/89.

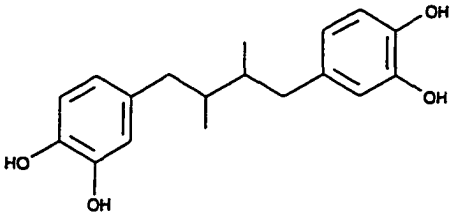
Judy Drake
Quality Assurance Technician

Product Information

Nordihydroguaiaretic Acid

Catalog No. 70300

CAS Registry No: 500-38-9
CA Index Name: 1,2-Benzenediol, 4,4'-(2,3-dimethyl-1,4-butanediyl)bis-
Synonyms: NDGA
MF: $C_{18}H_{22}O_4$
FW: 302.4
Purity: ≥95%
Stability: ≥1 year at -20°C
Supplied as: A crystalline solid
Melting Point: 186-187°C



Laboratory Procedures

For long term storage, we suggest that Nordihydroguaiaretic Acid (NDGA) be stored as supplied at -20°C. It will be stable for at least one year.

Concentrated stock solutions of NDGA can be prepared by dissolving the crystalline solid in an organic solvent such as ethanol, methanol, acetone, DMSO, or acetonitrile. The solubility of NDGA in these solvents is at least 100 mg/ml. Stock solutions prepared in oxygen-free solvents will be stable for at least one week if protected from light and stored at 0-4°C. Further dilutions of the stock solution into aqueous buffers or isotonic saline should be made prior to performing biological experiments. Also, ensure that the residual amount of organic solvent is insignificant, since organic solvents may have physiological effects at low concentrations. We do not recommend storing the aqueous solution for more than one day.

NDGA is a lipoxygenase inhibitor, as well as, an inhibitor of peptido-leukotriene biosynthesis. NDGA inhibited ionophore A23187-induced peptido-leukotriene biosynthesis in rat peritoneal cells with an IC_{50} of 5-7 µM.[1] NDGA inhibited electron transport in ascites tumor mitochondria at the NADH-dehydrogenase-ubiquinone level.[2]

References

1. Hope, W.C., Welton, A.F., Fiedler-Nagy, C., *et al. In vitro* inhibition of the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. *Biochem. Pharmacol.* 32, 367-371 (1983).
2. Pavani, M., Fones, E., Oksenberg, D., *et al.* Inhibition of tumoral cell respiration and growth by nordihydroguaiaretic acid. *Biochem. Pharmacol.* 48, 1935-1942 (1994).

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