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EVALUATION OF HUMAN RESPIRATORY MUSCLE FATIGUE

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This thesis is submitted to the Faculty of Graduate Studies and Research, in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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To my dear wife Yanlin

and daughter Roro

ABSTRACT

The first part of my work evaluates bilateral supramaximal transcutaneous phrenic nerve stimulation as a diagnostic test for respiratory muscle fatigue. I found that twitch transdiaphragmatic pressure (Pdi,T) was inversely and linearly related to lung volume (V_L) both before and after fatigue. Although fatigue caused significant decrease in PdiT amplitude at all V_L, the fractional decrease in Pdi,T was greater at high V_L, indicating the importance of V_L as an independent variable that needs to be controlled whenever Pdi,T is determined. Twitch mouth pressure (Pm,T) was found to be linearly related to twitch esophageal pressure (Pes,T), to Pdi,T, and to V_L. All these relationships were reproducible. Diaphragmatic fatigue resulted in significant decrease in Pm,T proportional to the decrease in Pdi,T for a given V_L so that Pm,T-Pes,T and Pm,T-Pdi,T relationships were unchanged. Thus the Pm,T-V_L relationship can be used to assess diaphragmatic fatigue non-invasively. Paired phrenic nerve shocks which were well tolerated by normal subjects can be used to obtained a measure of the pressure-frequency curves of the diaphragm, which were reproducible. In particular, I showed that the pressure ratio of diaphragmatic twitch elicited by the second shock at 10Hz over that at 100Hz ($T2_{10/100}$) is a valuable index of low frequency fatigue.

In the second part of my work I studied the effect of respiratory muscle fatigue on ventilatory response to CO_2 and respiratory muscle recruitment. The data showed that ventilatory response and respiratory muscle recruitment patterns were different

in a number of aspects between diaphragmatic fatigue and global inspiratory muscle fatigue. After diaphragmatic fatigue, the only change was an increase in the recruitment of rib cage muscles, which fully compensated for decreased diaphragmatic contractility because all the ventilatory parameters were constant. After global fatigue, both the diaphragm and rib cage muscles contributed less to breathing but expiratory muscles were recruited resulting in a decrease in end-expiratory P_L and an increased contribution of elastic energy stored within the respiratory system to inspiratory tidal volume generation. In spite of this, rapid shallow breathing developed while minute ventilation remained constant. These data suggest that the ventilatory control system can detect fatigue and has sufficient plasticity to alter inspiratory drive appropriately. The overall ventilation level can thus be maintained.

RESUME

La première partie de mon travail consistait à évaluer le potentiel diagnostique que la technique des stimulations bilatérales phréniques, supramaximales et transcutanées, peut avoir dans l'étude de la fatigue musculaire respiratoire. Les résultats obtenus démontrent une relation inverse et linéaire entre l'amplitude de la secousse transdiaphragmatique (Pdi,T) et le volume pulmonaire (V_L), avant et après un protocole de fatigue. Malgré un effet réducteur significatif de la fatigue sur l'amplitude de PdiT à tout les V_L, la réduction fractionnelle de ce dernier paramètre est relativement plus grande lorsque les V_L sont élevés, indiquant donc l'importance de contrôler ce facteur indépendant lors de mesures de PdiT. L'amplitude de la secousse à la bouche (Pm,T) est linéairement proportionnelle à celle de la secousse mesurée à l'aide de ballonnet oesophagien (Pes,T), de la Pdi,T et du V_L. Chacune de ces relations sont reproductibles. La fatigue diaphragmatique résulte en une diminution significative de la Pm,T proportionnelle à la diminution de la PdiT pour un V_L donné, de façon à ce que les relations entre la Pm,T et la Pes,T, ainsi que la Pm,T et la Pdi,T sont inchangées. Il est donc possible d'utiliser la relation entre la Pm,T et le V_L pour évaluer la fatigue diaphragmatique de façon non-invasive. Bien tolérés par les sujets normaux, des doubles stimuli phréniques peuvent être utilisés pour l'obtention des courbes pression-fréquence du diaphragme, et ce, de façon reproductible. Les résultats indiquent que la relation entre les secousses transdiaphragmatique à 10 Hz et

100 Hz ($T2_{10/100}$) en réponse à la seconde stimulation phrénique d'un doublet est un indicateur valide de la fatigue à basse fréquence.

Pour la seconde partie de mon travail j'ai étudié l'effet de la fatigue des muscles respiratoires sur la réponse ventilatoire induite par le CO_2 et sur le recrutement de ceux-ci. Les résultats démontrent que la réponse ventilatoire et le recrutement des muscles respiratoires est, sous plusieurs aspects, différent pour la fatigue spécifiquement diaphragmatique ou pour la fatigue globale des muscles inspiratoires. Suite à la fatigue diaphragmatique, le seul changement observé est un recrutement accru des muscles de la cage thoracique qui compense complètement la baisse de contractilité du diaphragme puisque tous les paramètres ventilatoires restent constant. Suite à une fatigue de type globale le diaphragme et les muscles thoraciques contribuent de façon réduite à la respiration. Dans cette situation, les muscles expiratoires sont recrutés et causent une diminution de la pression transpulmonaire (P_L) à la fin de l'expiration. De plus, la contribution de l'énergie élastique du système respiratoire disponible à la production du volume courant inspiratoire se voit augmentée. Malgré ceci, un mode ventilatoire rapide et court apparait dans ces conditions et la ventilation minute reste constante. Ces résultats suggèrent donc que le système contrôlant la ventilation peuvent détecter la fatigue des muscles et qu'il existe à ce niveau une plasticité suffisante permettant de modifier le recrutement des muscles inspiratoires et le maintient d'un niveau de ventilation approprié.

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Firstly, I would like to express my deep appreciation to my thesis supervisor, Dr. Peter T. Macklem. Dr. Macklem not only provided me with the opportunity to pursue the research which was presented in this thesis, but also introduced to me the way of scientific thinking and reasoning. Dr. Macklem has made an important contribution to my future career as a scientist.

Next I would like to give my sincere thanks to Dr. Francois Bellemare. Dr Bellemare is the one that introduced me to the research of respiratory muscle mechanics and fatigue and was a co-supervisor for the first part of my research in Meakins-Christie Laboratories (chapters 2,3,4 of this thesis).

My thanks are also extended to Mr. Alain P. Gauthier, Dr. Thomas Similowski, Dr. Pawel Siwinski, Mr. Robert Faltus, Dr. Ioannis Lichros, Mr. Bob Thomson, Mr. Serge Filiatrault, and many others in Meakins-Christie Laboratories for their continuous help, patience, friendliness during the period that this work was performed.

Finally, I feel that I am in debt to my dear wife Yanlin for her understanding, support, and encouragement throughout the past three years during which this work was performed.

TABLE OF CONTENTS

CONTENTS PAGE

CONTENTS **PAGE**

LIST OF FIGURES

LIST OF TABLES

Prologue

The thesis is composed of seven chapters. Chapter 1 is a general review of the literatures which provides background information pertaining to the thesis. In chapter 2, 3, 4, 5, 6, I have taken the advantage of the option provided by section B.2 of the "Guidelines Concerning Thesis Preparation" which states:

> Candidates have the option, **subject to the approval of their Department**, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that these copies are bound as an integral part of the thesis. If this option is chosen, **connecting texts, providing logical bridges between the different papers**, are mandatory.
>
> -The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published. **The thesis must include, as separate chapters or sections:** (1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overal conclusion and/or summary.
>
> -Additional material (procedure and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.
>
> -In the case of manuscripts co-authored by the candidate and others, **the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent;** supervisors must attest to the accuracy of such claims at the Ph.D. Oral Defense. Since the task of the Examiners is made more difficult in these cases, it is the candidate's interest to make perfectly clear the resposibilities of the different authors of co-authored papers.

Chapter 2 has been published in the Journal of Applied Physiology 1992;72:1064-1067; chapter 3 has been published in the

American Review of Respiratory Disease 1992;145:1064-1069; chapter 4 has been accepted for publication and is in press in the European Respiratory Journal; chapter 5 and chapter 6 have been submitted to the Journal of Applied Physiology and are under revision; chapter 7 contains conclusion and claims for originality.

It is difficult to study humans in vivo alone. For these experiments presented in chapter 2 through chapter 6, I received technical assistance from a number of research fellows in the Meakins-Christie Laboratories. These include Mr. Alain P. Gauthier, Dr. Thomas Similowski, Dr. Pawel Sliwinski, Dr. Ioannis Lichros. Mr. Robert Faltus prepared the rat diaphragmatic strips for part of the experiment presented in chapter 4. Their assistance was only during the experimental procedures. They did not participate in experimental design, data analysis and interpretation.

Dr. Peter T. Macklem, my thesis supervisor, is a co-author for all the manuscripts of this thesis. Dr. Francois Bellemare is a co-supervisor and co-author for papers presented in chapter 2, 3, 4. Mr. Alain P. Gauthier is a co-author for manuscripts comprising chapter 2, 3, 4, and 6. Dr. Thomas Similowski is a co-author for manuscripts comprising chapter 2, 3, and 4. Drs. Pawel Sliwinski and Ioannis Lichros are co-authors for manuscripts presented in chapter 5 and 6. Mr. Robert Faltus is a co-authors for manuscripts of chapter 4.

Appendix 1 gives International System of Units (S.I. Units) equivalents. Appendix 2 contains the list of abbreviations used in this thesis. Appendix 3 contains my academic research record.

CHAPTER 1

INTRODUCTION

1.1 Important Concepts of Respiratory Muscle Fatigue.

1.1.1 History

The respiratory pump, like the heart, is vital. Pump failure of the myocardium causes circulatory failure. Similarly, pump failure of the respiratory muscles may cause ventilatory failure (226).

The importance of respiratory muscle failure for ventilatory failure was recognized early in this century. Davies et al (58) demonstrated that human subjects adapt to large inspiratory resistances by rapid shallow breathing, which was attributed to central respiratory muscle fatigue. Davies et al (57) then showed that the ventilatory response to CO_2 was limited at very high CO_2 drive possibly due to peripheral fatigue of respiratory muscles. Later, understanding of skeletal muscle physiology greatly stimulated the research related to respiratory muscles. From the 1940s to 1960s, the fiber type composition (56,104) and contractile properties (106,218) of various respiratory muscles from different species have been described. During the same period, pressure-volume relationships of in vivo human respiratory system during relaxation and during voluntary inspiratory and expiratory efforts were reported in detail by Rahn et al (213). On the basis of this work, the measurement of maximal inspiratory pressure ($P_{I,max}$) (29,37,47) was developed and it was noticed that it was decreased in patients with ventilatory insufficiency and weaning difficulty from mechanical ventilation (230,231). Agostoni and Rahn (5) made the first measurements of transdiaphragmatic pressure (Pdi) as

pressure difference between the pleural space and abdomen. They recorded a Pdi of about 100cmH_2O in normal subjects during a maximal voluntary inspiratory efforts against an occluded airway.

Although Agostoni and Fenn (3) had speculated that force-velocity characteristics of respiratory muscles could possibly be a factor for airflow limitation and Freedman et al (99) suspected that the physical performance of athletes might be limited by respiratory muscle fatigue during exercise that required maintenance of high levels of ventilation, traditional concepts generally prevailed that ventilatory failure was to a large extent a direct result of deterioration of lung function or abnormalities in ventilatory control. The inspiratory pump was not implicated because it was believed that the respiratory muscles had sufficient reserves to meet the ventilatory demands (213). In 1977, Roussos and Macklem (226) in their study of normal human subjects showed that fatigue of the diaphragm (which behaves similarly to other skeletal muscles), could be induced if the inspiratory load was sufficiently high and breathing against such a load was sufficiently long. Based on these experimental results, they further proposed that ventilatory failure may occur as a result of respiratory muscle fatigue (226). Since then, extensive research has been accomplished in this field and great progress has been achieved in the past 15 years. This research comprises the major part of our current understanding of respiratory muscle function and fatigue. In spite of this, the prevalence and importance of inspiratory muscle fatigue in ventilatory failure remain unknown

due to the lack of a readily applicable clinical test to establish a diagnosis of fatigue.

1.1.2 Definition of fatigue

Skeletal muscle fatigue has been traditionally defined as a failure of the muscle to generate and maintain a required or expected force (75,77). The emphasis has been on the decrease in the ability for the muscles to develop force with fatigue since force generation is easy to measure. Just as important, however, are the ability to change length and produce velocity of shortening. In addition, there are other alterations with fatigue such as the changes in the muscle EMG power spectrum and the slowing of relaxation (77). Because respiratory muscles are thought to have similar physiological characteristics as other skeletal muscles, the same definition of fatigue was used for respiratory muscles (194,227,228).

There are three major drawbacks of this definition. First, a muscle is not only able to generate force but also to shorten with a certain velocity against a load that requires a force smaller than its maximal isometric force. The shortening of a muscle produces displacement, which is particularly important for respiratory muscles, the primary function of which is to generate lung volume changes by shortening. It has been shown (220) that inspiratory muscle fatigue causes a decrease in both the amount of and the velocity of shortening equivalent to that seen in other skeletal muscles. In addition, as shown by Mardini and McCarter in an *in vitro* rat diaphragmatic strip model (169), the ability to

generate isometric force and the ability to shorten do not always decline to the same extent following fatigue. The degree of impairment of these separate actions is dependent on the type of fatigue protocol and the magnitude of load. Thus the changes in one do not necessarily mirror the changes in the other. Secondly, the functional and metabolic changes of fatigue develop during the first few contractions against a fatiguing load and this process continues until the maximal force of the muscle has declined to the point below the target (111,217). At this point task failure occurs. According to the old definition fatigue is not present until task failure happens. Yet clearly, important muscle mechanical and metabolic changes occur long before task failure. Thirdly, the functional alterations of a muscle as a result of fatigue differ from those changes secondary to other conditions such as neuromuscular disease which may result in weakness but, unlike fatigue, do not recover with rest. The unique effect of rest in restoring function to a fatigued muscle should be incorporated into the definition of muscle fatigue.

Accordingly, the National Heart, Lung and Blood Institute (NHLBI) workshop (217) held in 1988 agreed that skeletal muscle fatigue can be defined as a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest. Muscle weakness was defined as a condition in which the capacity of a rested muscle to generate force is impaired. This latter condition includes the loss of force of respiratory muscles

as a result of the changes in muscle resting length, disuse atrophy, nutritional status, neuromuscular diseases and other disease states (111,194).

Although the loss of force is not the only alteration produced by muscle fatigue and task failure is not a synonym of fatigue according to the new definition, changes in force or pressure are currently the most frequently employed parameters to detect respiratory muscle fatigue. This is especially the case for the study of humans because this measurement is easily accessible, and task failure is still an important criterion for experimentally induced-fatigue.

In a broad sense, respiratory muscle fatigue should include both inspiratory and expiratory muscle fatigue. In practice, however, respiratory muscle fatigue is usually used as a synonym for inspiratory muscle fatigue because expiration is generally considered as a passive process or, when active, only requires a small percentage of the force available. However, decreased maximal expiratory pressure has been demonstrated in one study in which the subjects performed expiratory resistive loaded breathing to fatigue their expiratory muscles (243). In the present work, the expression "respiratory muscle fatigue" only refers to the fatigue of inspiratory muscles.

1.1.3 Endurance and tension-time index

Experimental fatigue of respiratory muscles is generally induced by having the subject breathe in through a high inspiratory resistance and maintain a target inspiratory pressure in a square

wave form that is a predetermined fraction of the subjects' maximal capacity until the target can no longer be achieved or maintained. Expiration is generally unloaded. The elaspsed time between the beginning of loaded breathing and the point of task failure is called endurance time (Tlim).

Using Pdi as the target, Roussos and Macklem (226) demonstrated that in normal subjects, diaphragmatic endurance Tlim was inversely and curvilinearly related to Pdi/Pdi,max ratio during the fatigue task; with this ratio greater than 0.4, diaphragmatic fatigue would sooner or later occur while Tlim tended to infinity when Pdi/Pdi,max was equal or less than 0.4. Using a similar approach but with the mouth pressure (Pm) instead of Pdi as a target they induced global inspiratory muscle fatigue or fatigue of all inspiratory muscles, in contrast to selective diaphragmatic fatigue (225). They showed that the critical value for Pm/Pm,max that could be sustained indefinitely was approximately 0.6 (225). Based on these results, they proposed (225,226) that inspiratory muscles, like other skeletal muscles, can be fatigued if the load on the muscle is high enough; they suggested that if respiratory muscle fatigue occurs clinically because of increased breathing load, decreased strength, or a decreased energy supplies, the consequence would be ventilatory failure.

For skeletal muscles, endurance is dependent on the length of the contraction phase relative to the relaxation phase, or duty cycle (the ratio of contraction time to the sum of contraction and relaxation times) (224). During relaxation the exercising muscle

rests and its blood supply may increase. Roussos and Macklem (226) recognized the importance of the pressure time product and used it to compare endurance time of the diaphragm under normoxic and hypoxic conditions. Bellemare and Grassino (21) formally identified the role of duty cycle in determining endurance time in normal subjects and clearly demonstrated that diaphragmatic Tlim was critically dependent on the product of Pdi/Pdi,max and duty cycle (Ti/Ttot) which they called the tension-time index of the diaphragm (TTdi). Although Roussos and Macklem had shown theoretically that this approach was valid only over specified limits (226), it has nevertheless proven to be satisfactory to characterize diaphragmatic Tlim over considerable variations of tidal volume and inspiratory flow rates. It has been established (21) that a TTdi smaller than 0.15 is usually associated with an infinite Tlim and a TTdi greater than 0.15-0.20 usually leads to a finite Tlim and task failure within 45 min. Accordingly, the TTdi 0.15-0.20 is named the critical TTdi and the zone covered by TTdi 0.15-0.20 in the Ti/Ttot vs. Pdi/Pdi,max plot (21,23,110) is called fatigue threshold or fatigue zone.

Compared with normal subjects breathing quietly with a TTdi of about 0.02 (21), a group of severe but stable patients with chronic obstructive pulmonary disease (COPD) had a mean TTdi of 0.05 (range 0.01-0.12) at rest (23). The increase in TTdi in these patients mainly resulted from the increase in Pdi/Pdi,max secondary due to decreased Pdi,max and/or increased tidal Pdi swing due to high airway resistance. Furthermore, when a breathing pattern was

imposed by increasing Ti/Ttot from 0.33 to 0.49 so as to increase TTdi from 0.07 to 0.17, EMG signs of diaphragmatic fatigue (116) appeared. The patients were unable to tolerate such a change in breathing pattern for long (23). Similarly, Pardy and Roussos (208) showed in 6 COPD patients that voluntary hyperventilation of a magnitude that caused no fatigue in normal controls, increased the Ppl/Ppl,max ratio from about 0.23 to 0.44 and led to EMG evidence of diaphragmatic fatigue. These results were interpreted as the evidence that fatigue threshold of the diaphragm in COPD patients is similar to normal subjects and the force reserve of the diaphragm in COPD patients is considerably reduced compared with normal subjects (23). This is in agreement with the assumption that these patients may be at particular risk to develop respiratory muscle fatigue and resultant ventilatory failure (225,226).

It is difficult to determine the fatigue threshold of rib cage muscles since the tension-time index of rib cage muscles (TTrc) derived from the pleural pressure-time integral is only an index of the pressure developed by these muscles and the muscles are lumped as if they are a single group. The tension, shortening, and timing of contraction of these muscles can be quite different, one from another. As a result, the fatigue threshold could be different for each muscle. Normal subjects performing rib cage inspirations against an inspiratory resistive load while sparing the diaphragm as much as possible demonstrated that the fatigue threshold of TTrc was below 0.26 for both the parasternal intercostal and sternocleidomastoid muscles (97). This is not too far from the

fatigue threshold of the diaphragm (TTdi 0.15-0.20) as reported by Bellemare and Grassino (21). However, these data (97) must be regarded as an approximation because until very recently no methods have been available to measure the pressures developed by the rib cage inspiratory muscles during breathing, and even now there are no methods to measure the maximal pressures they develop (255).

Bellemare and Grassino (22) have also shown that the rate of decay in diaphragmatic EMG high-to-low frequency ratio indicating fatiguing contraction of the diaphragm (116) was tightly correlated to TTdi when it was above 0.15-0.20 independent of the different combinations of Pdi/Pdi,max and Ti/Ttot. What are the underlying mechanisms that determine the fatigue threshold of a TTdi of 0.15-0.20? Roussos and Macklem and associates (225,226) hypothesized that diaphragmatic fatigue occurs when the rate of energy consumption exceeds the rate of energy supplies. In support of this hypothesis, the rate of O_2 consumption during inspiratory resistive loads is positively related to TTdi (96) and inversely related to Tlim (175). In dogs, diaphragmatic blood flow is increased with increasing TTdi up to 0.20; thereafter a further increase in TTdi is associated with a progressive decrease in the blood flow to the diaphragm (24). Thus, when the load against which the respiratory muscles contract is increased and more energy is consumed, respiratory muscle contraction itself may limit further increase in the blood flow and energy supply. Under these circumstances blood flow supplies energy largely during relaxation, indicating the importance of the duty cycle. For a given task weak muscles demand

more energy relative to that expended at maximal power output than strong ones, so that increased load, decreased strength, increased duty cycle, and decreased energy supplies all lead to an imbalance between supply and demand leading to respiratory muscle fatigue.

How energy supplies (cardiac output, SaO_2, Hb concentration, distribution of blood flow, etc.), muscle length (lung volume), and velocity of shortening (inspiratory flow) influence the actual values of TTdi remains largely unexplored. It is possible that the critical TTdi can vary considerably. A number of studies have now shown that tension-time index may not accurately predict fatigue of respiratory muscles in all circumstances. For instance, diaphragmatic fatigue became evident during severe hypotension with a TTdi that does not induce fatigue under normotensive conditions (122), indicating that the critical TTdi is lower than 0.15-0.20 when energy supplies are diminished; diaphragmatic fatigue of normal subjects with a restricted rib cage occurs during heavy exercise with a TTdi of only 0.06 (123), considerably less than the critical value of TTdi 0.15-0.20. This may possibly be explained by the high velocity of diaphragmatic shortening under this experimental condition. Indeed, when alterations in tidal volume and flow rates were greater than those explored by Bellemare and Grassino (21), TTdi was less accurate in predicting the occurrence of diaphragmatic fatigue (44,73,174). The critical value of TTdi for diaphragmatic fatigue also becomes significantly smaller than 0.15-0.20 with hypercapnia (133), a condition frequently encountered in patients with pulmonary disorders. In spite of these

observations, the tension-time index remains a useful predictor of fatigue and endurance time in normal subjects breathing at normal lung volumes with normal energy supplies and contraction velocities that are not excessive.

1.1.4 Site and type of respiratory muscle fatigue

From a theoretical point of view, respiratory muscle fatigue can occur as a result of impairment at any site from the brain cortex down to the peripheral muscle machinery. Conventionally, skeletal muscle fatigue is thought to have two major origins: a central origin which when applied to respiratory muscles refers to a reduced motor drive arising from cortex, brainstem respiratory centres or respiratory motoneurons failing to maintain muscle activation (central fatigue), and a peripheral origin which describes reduced muscle performance because of insufficient response of the muscle to a normal or an augmented central activation (28). This can occur at the motor end plate, sarcolemmal membrane, t-tubules, sarcoplasmic reticulum or in the contractile machinery itself.

Central fatigue can be detected by superimposing an external stimulation on ongoing maximal voluntary contraction of a muscle. If the voluntary contraction is already maximal, superimposed external stimulation should not elicit any additional force. If additional force can be generated by the artificial stimulation, the "maximal voluntary contraction" must have been in fact submaximal; central fatigue is then suspected.

The role played by central fatigue in the pathophysiology of

skeletal muscle fatigue is still controversial. Merton (182) has shown many years ago that maximal voluntarily generated and maximal stimulated forces of the adductor pollicis muscle are the same. Similarly, it has been demonstrated that both normal subjects (17,101) and patients with chronic airflow limitation (204) are able to maximally activate their diaphragm. However, as shown by Bigland-Ritchie and coworkers (26) on quadriceps muscle, although central fatigue may not occur during short or intermittent contractions, it may contribute to the loss of force of the muscle during sustained efforts. In respiratory muscles, by using phrenic nerve stimulation superimposed on voluntary diaphragmatic contraction during inspiratory resistive loaded breathing in humans, Bellemare and Bigland-Ritchie (18) estimated that as Tlim is approached, central fatigue roughly accounts for 50% of diaphragmatic force loss. It is possible that the central component results from reflex inhibition of motoneuron firing frequencies by activating afferent neurons within the fatiguing muscle.

The results from animals are also conflicting. In an <u>in situ</u> dog model of cardiogenic shock induced by cardiac tamponade, Aubier and associates (14) found that Pdi increased in the first hour after shock but progressively decreased thereafter until death in spite of a significant increase in the amplitude of diaphragmatic EMG throughout the experiment, suggesting that the diaphragmatic fatigue in these dogs was peripheral in origin. In contrast, in dogs with hemidiaphragmatic fatigue induced by phrenic artery occlusion, there was a significant decrease in the diaphragmatic

EMG activity on the affected side which paralleled the decrease in force; at the same time the EMG activity from the contralateral hemidiaphragm and from rib cage inspiratory muscles was increased. These changes returned to baseline upon reperfusion of the occluded phrenic artery, suggesting central diaphragmatic fatigue (241). There have been other animal studies in favour of central origin (203), of peripheral origin (122,125), or of both origins (220) as the site of respiratory muscle fatigue.

It is difficult to interpret the conflicting results from these studies. In particular, it is sometimes very hard to separate central fatigue from lack of cooperation and motivation in some subjects. The available information does not provide direct, clear-cut, and sufficient evidence of central fatigue as an important contributor to human skeletal muscle fatigue. Whether central fatigue occurs or not may depend on the fatigue protocol, type of load, the muscles involved, and other experimental variables. The argument of central versus peripheral fatigue as if it was an either/or condition may be misplaced. The hypothesis that peripheral fatigue leads to central reflex inhibition of motoneuron firing frequencies seems an appealing one.

Since most of the studies exploring central fatigue have been carried out on subjects or animals that were "normal" prior to the tests, absence of evidence of central fatigue does not exclude the possibility that central fatigue may exist in patients. In this connection, patients with difficulty in weaning from mechanical ventilation, when breathing on their own, increase breathing

frequency early in the weaning trial followed by a decrease in breathing frequency and minute ventilation at the end in spite of an elevated $PaCO_2$ and a rapid fall in arterial pH (46). This mimics the fall in frequency observed in dogs with experimental cardiogenic (14) or septic shock (125) which occurs immediately prior to death. This remarkable alteration in breathing frequency clearly indicates an important influence of the experimental conditions on central control of breathing. Other examples which support that the physiological conditions in which the respiratory muscles operate can influence central control of breathing include the observations that: during inspiratory resistive loaded breathing to exhaustion, most stable COPD patients show parallel decreases in both Pdi and diaphragmatic EMG amplitude (Edi) leading to an unchanged or even elevated Pdi/Edi ratio (141); some patients with hypoventilation and CO_2 retention do not normalize arterial CO_2 by voluntarily increasing ventilation though they have the capacity to do so presumably because augmenting ventilation will put the tension-time index of their respiratory muscles above the fatigue threshold (16,23). It is of practical importance to know whether the brainstem respiratory centres drive the respiratory muscles to exhaustion (peripheral fatigue) in order to maintain ventilation or whether they adapt and maintain drive constant or even decrease the drive to the respiratory muscles (central fatigue) at the cost of hypoventilation when the load of breathing is increased in patients. The clues described above from patient studies seem, though indirectly, in favour of the latter strategy.

Despite the many uncertainties and controversies concerning central fatigue, it is now evident that excessive work of skeletal muscles including the respiratory muscles can induce peripheral fatigue, and that this occurs with impairment beyond the neuromuscular junction (130). Two major types of impairment have been documented for peripheral fatigue (28,76,77,111,194,227,228): [1] neuromuscular transmission or muscle membrane action potential propagation failure (which will be referred to as transmission failure) featured by a decrease in the size of muscle action potentials and a resultant reduction in force for a given activation and; [2] muscle excitation-contraction coupling failure characterized by decrease in force with well maintained muscle action potentials for a given activation. The former is thought to result from an excessive accumulation of potassium extracellularly, particularly in the t-tubules and sodium intracellularly, while the latter is possibly due to impaired release of calcium from the sarcoplasmic reticulum.

In 1932, Davis and Davis (59) reported that when the soleus and gastrocnemius muscles were electrically stimulated to fatigue, the maintenance of force was best achieved by stimulating the muscles with relatively low frequencies compared with high frequencies. The smaller force elicited by high frequencies could partially recover if the muscles were stimulated at a lower rate. Furthermore, the action potentials recorded from the fatigued muscles were smaller the higher the stimulation rate. This observation was later supported by Krnjevic and Miledi (145) in

in vitro and *in situ* and further extended by Stephens and Taylor (239) *in vivo*. These latter authors showed that during a sustained maximal voluntary contraction there was a consistent decline in the size of muscle action potentials in response to high frequency stimulation. All these studies indicate that the loss of force during high frequency stimulation is attributable to impairment of effective transmission or propagation of muscle action potentials. In other words, this type of fatigue reflects activation failure at high frequencies.

In contrast, working on adductor pollicis muscle, Merton (182) and Bigland-Ritchie et al (27) failed to show the attenuation in the size of evoked muscle action potentials during a sustained maximal voluntary contraction when maximal force had substantially reduced suggesting that transmission failure was not responsible for the loss of force observed. This conclusion was contested recently by Bellemare and Garzaniti (20) who showed that decrease in the size of action potentials indicating transmission failure could be detected with a tetanus during sustained maximal voluntary contraction but not with single shocks as used by Merton (182) and Bigland-Ritchie et al (27). However, Merton and coworkers (183) also demonstrated that massive electrical stimulation (around 300 volts) of the motor cortex did not reveal a noticeable decline in the size of evoked action potentials on adductor pollicis muscle at the time the sustained maximal voluntary force had significantly declined indicating intact functioning of the pathway from cortex to muscle. Furthermore, the decrease in sustained maximal voluntary

force was not restored by massive direct stimulation (up to 2400 volts) to the contracting muscles indicating a failure in the contractile machinery itself.

A number of studies have been carried out to evaluate the contribution of transmission failure to respiratory muscle fatigue. The contribution of transmission failure to overall force loss during diaphragmatic fatigue induced by repetitive electrical stimulation can be estimated by comparing the forces elicited by phrenic nerve stimulation and by direct muscle stimulation, assuming that the decrease in force developed by direct muscle stimulation during fatigue reflects the failure in muscle contractile machinery. Kelsen et al (135) demonstrated in rats that at the end of 6 min of repetitive diaphragmatic stimulation at 15Hz, the force of the diaphragm generated by nerve stimulation became progressively less than that generated by direct muscle stimulation and estimated that transmission failure contributed about 18% of total loss of force. Similar results were reported by Massarelli et al (172) when the rat diaphragm was fatigued by repetitive stimulation at 26Hz. However, Aldrich and associates estimated that transmission failure accounted for up to 82% of the decrease in diaphragmatic force in rats using a similar approach (8) and almost all the fall in diaphragmatic force in rabbits <u>in situ</u> with fatigue induced by inspiratory resistive loaded breathing (7). Such a big discrepancy of the results between these studies can not be simply explained by interspecies differences. The results of subsequent work of Kuei and colleagues (146) were in

agreement with those of Kelsen et al (135) and Massarelli et al (172) and argued against those of Aldrich and coworkers (7,8). They demonstrated that transmission failure reached a maximum at 75Hz and contributed 43% of the overall force decrement of the rat diaphragm, suggesting that the major impairment resulting from respiratory muscle fatigue is at the level of contractile machinery. These authors also suggested that the overestimation of transmission failure by Aldrich (8) resulted partly from the low Ca^{2+}/Mg^{2+} concentration and the high temperature in the muscle bath. It needs to be pointed out that because of the technical difficulty of in vivo investigation in humans, the studies to date of transmission fatigue of respiratory muscles have only been performed on animal preparations with fatigue induced in the majority of cases by repetitive stimulations. The results derived from these studies can not be directly employed to infer the characteristics of respiratory muscle fatigue in humans, especially in patients. For instance, available information from rats in vitro indicates that diaphragmatic transmission failure only becomes evident when stimulation frequencies reach very high levels (146). However, the firing frequencies of the phrenic motoneurons during normal breathing are seldom over 30Hz (75,131). Furthermore in in vitro experiments all motor units are activated synchronously. This never occurs under physiological conditions. It seems reasonable to postulate that transmission failure of respiratory muscles, if present, may be far less important than failure of the excitation-contraction coupling process.

When the force of skeletal muscles is developed and recorded at different stimulation frequencies, force-frequency relationships can be measured. By measuring the rate of force recovery, peripheral fatigue of skeletal muscles can be divided into high and low frequency types referred to as high frequency fatigue and low frequency fatigue, respectively (75-77). High frequency fatigue occurs at high stimulation frequencies and recovers quickly in minutes. This type of fatigue can be seen in patients with myasthenia gravis and when the muscle is cooled and is thought to result at least partly from transmission failure; in low frequency fatigue there is a selective loss of force at low stimulation frequencies which recovers very slowly over hours. It generally develops following intense dynamic and static muscular work, and is attributed to failure of excitation-contraction coupling or of the contractile machinery (75-77).

Edwards et al (81) established the force-frequency relationships of human adductor pollicis and quadriceps muscles with fatigue induced by submaximal and maximal voluntary and stimulated contractions. They showed that the shape of the force-frequency curves of both muscles are characteristic; the force quickly increased between 10Hz-30Hz and reached a plateau at higher frequencies between 50Hz-100Hz. Their fatigue protocol produced a more significant decrease in force when measured as a twitch or at a low rather than at a high stimulation frequency. The force developed at 100Hz recovered to over 90% of initial value at 30min while the force recorded at 20Hz remained low for up to 24 hours

with fully preserved muscle action potentials indicating an intact activation. This study clearly showed the long time course of recovery of low frequency fatigue and suggested that because of the distinct rate of force recovery at different stimulation frequencies, a sustained depression of force ratio at a lower frequency (10Hz-20Hz) to that at a higher frequency (50Hz-100Hz) could be used to infer low frequency fatigue. This work also emphasized the fact that since the motoneuron firing frequencies in daily activities are over the steep portion of force-frequency curves and within the low frequency range, the long-lasting nature of low frequency fatigue must have a major effect on daily activities if there is failure to recruit a larger number of motor units and/or to increase the mean firing frequency of the motoneurons (central fatigue).

Force-frequency relationships of human respiratory muscles has been established in the diaphragm using phrenic nerve stimulation (11,15,19,196,198) and in the sternomastoid muscle with a stimulating electrode directly placed on the muscle surface (197,198). These studies suggest that the shape of force-frequency curves of the respiratory muscles is very similar to that reported for other skeletal muscles (81). It has also been demonstrated that (as has been observed in other skeletal muscles), inspiratory resistive loaded breathing to exhaustion preferentially reduces force development of both the diaphragm and the sternomastoid at low stimulation frequencies with delayed recovery, causing the rightward displacement of the force-frequency curves and a

decreased low to high frequency force ratio (11,82,195-198). Low frequency fatigue of the sternomastoid muscle was also associated with a decreased muscle endurance (82). Finally, low frequency fatigue of the sternomastoid muscle (83,247) has also been observed in a small number of COPD patients breathing against an increased inspiratory load.

The observations from normal subjects as well as from patients indicate that excessive work of inspiratory muscles against an increased load can produce low frequency fatigue of the respiratory muscles. It has to be admitted that most of the observations of respiratory muscle low frequency fatigue were obtained in a laboratory environment with externally added loads. To what extent these findings represent the true picture in patients with increased intrinsic loads of breathing due to respiratory illness remains to be further investigated. To accomplish this goal, however, requires an objective, reliable, reproducible, specific, sensitive, easy and safe clinical diagnostic procedure to be developed for the detection of respiratory muscle fatigue in patients.

1.1.5 Contractile properties of major inspiratory muscles

Contractile properties describe muscle physiological function during contraction. They include peak isometric twitch and tetanic tension, twitch/tetanus ratio, the time to peak tension and the rate of the change in tension during contraction and relaxation, and active force-length and force-velocity relationships.

To begin with, skeletal and respiratory muscle contractile

properties are critically dependent on the composition of muscle fiber types. Extensive work has been done in this field for skeletal muscles including the respiratory muscles. Briefly, according to the work of Engel (84), Brook and Kaiser (35), Peter et al (211), and of many others, fiber type composition of skeletal muscles can be divided into slow-twitch oxidative (SO or type I), fast-twitch oxidative glycolytic (FOG or type IIa), and fast-twitch glycolytic (FG or type IIb). SO fibres have a poorly developed glycolytic enzyme system, a low activity of myofibrillar ATPase and a well developed oxidative enzyme system. They have a long isometric twitch time, a slow shortening velocity, and a high twitch/tetanus ratio. Their maximal tension is relatively small but SO fibres are well designed to resist fatigue. FOG fibres have well developed oxidative and glycolytic enzyme systems and high myosin ATPase activity. These fibres have a short twitch time, generally a fast velocity of shortening, a low twitch/tetanus ratio, and a reasonable resistance to fatigue. FG fibres have a well developed glycolytic enzyme system and high activity of myosin ATPase but very low oxidative enzyme potential. FG fibres have short twitch time, fast shortening speed, low twitch/tetanus ratio, high maximal tension, but the least resistance to fatigue.

Most of the skeletal muscles have a mixed fiber type composition and their contractile properties depend on the predominant fiber type. For example, a muscle with a great majority of fibers being SO will behave as a slow muscle and that with predominant FG and FOG a fast muscle. Respiratory muscles also

contain all three fiber types described. The diaphragm is the major inspiratory muscle and was therefore received more detailed investigation than other respiratory muscles. It was found that the diaphragm is intermediate in its fiber type composition between a typical slow muscle and a typical fast muscle (56,104,152,176,184). The fiber type composition of the human diaphragm, according to most authors quoted, is 55% SO, 21% FOG, and 24% FG (152). The data for other inspiratory muscles are relatively scanty. Greer and Martin (114) suggested that cat parasternal intercostal muscles have 55% SO, 22% FOG, and 23% FG. Mizuno and Secher (189) identified 59-64% SO, 18-27% FOG, and 12-23% FG fibers in human parasternal intercostal muscles, which is not very different from the diaphragm.

According to Lieberman et al (152), since respiratory muscles have mixtures of fiber types and fatigue resistance is different in different fibers, the endurance may reflect the net sum of the fatigue processes developed in each fiber type and to a large extent represent the contribution of the SO, non-fatiguing fiber population. This seems true in guinea pig diaphragm fatigued by repetitive stimulation and in humans during maximal voluntary ventilation (152). Whether or not this is the case for sustainable load of inspiratory muscles (225,226) has not been directly confirmed. Keens et al (134) reported that chronic high respiratory loads increased the SO fiber components in rat diaphragm. Farkas and Roussos (92) demonstrated that elastase-induced emphysema with endurance training increased oxidative capacity of hamster

diaphragmatic fibers but did not change the fiber type composition.

It has been recognized that muscle mechanical performance is determined by its length-tension and force-velocity characteristics. The force a muscle generates is critically dependent on the length of its fibres when it contracts (figure 1.1). This property is described by the muscle's length-tension relationship. Muscles generate maximal isometric force at optimal or resting length which is generally the length at which passive tension develops. When the muscle shortens, the force it develops is progressively reduced until the muscle ceases to generate force. If the muscle is stretched beyond its optimal length, passive tension increases but active force is again reduced. This length-tension relationship has been explained by Gordon et al (109) based on the sliding filament theory. The force developed depends on the extent of overlap between the thin and thick filaments, which in turn is determined by sarcomere length. By correlating length-tension data with microscopically observed sarcomere length and filaments overlap, these authors described in frog muscle six stages by which geometrical relation between thick and thin filaments could explain length-tension relationship (109): [1] sarcomere length is 3.65μm corresponding to the sum of thick (2.05μm) and thin (1.60μm) filaments; active tension is zero at and beyond this length because these is no overlap between thick and thin filaments; [2] active tension is almost linearly increased with decreasing sarcomere length from 3.65μm to 2.25μm due to increased overlap between thick and thin filaments. It is assumed

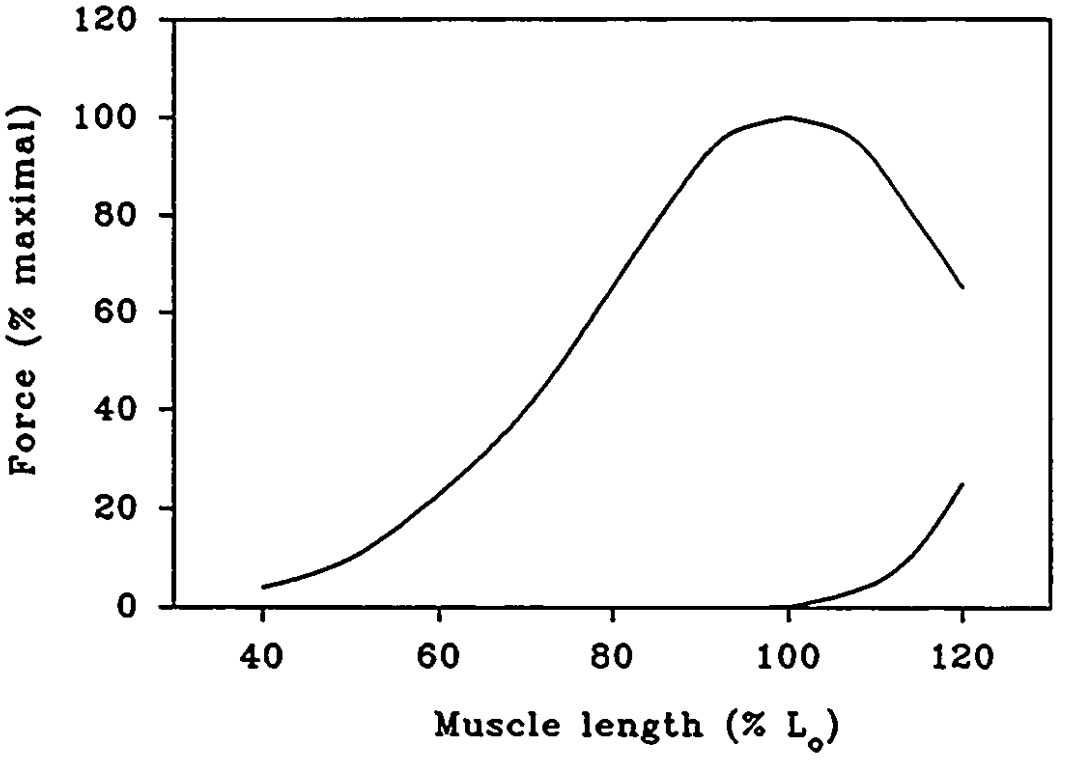

Figure 1.1: Schematic active and passive length-tension relationships of skeletal muscle. Muscle length is expressed as % optimal length (L_o) and force is expressed as % maximal active force.

that at this stage there is a constant tension generation per cross-bridge formation and a uniform distribution of the cross-bridge along the thick filament. The tension is maximal at sarcomere length 2.25μm corresponding to a maximal overlap of the thick and thin filaments; [3] the tension remains maximal at a sarcomere length between2.05μm and 2.25μm with the number of cross-bridge formation relatively constant; [4] and [5] when sarcomere length is shorter than 2.05μm, active tension is progressively decreased, the slope of the descending tension curve is steeper at sarcomere length shorter than 1.67μm than between 1.67μm and 2.05μm, which was believed to be due to collision of the ends of thick filaments with the Z lines resulting in blockade of cross-bridge activity; [6] active tension is again zero at sarcomere length 1.27μm where the thick and thin filaments attachment is completely ineffective because further overlap of thin filaments is blocked by the Z bands (figure 1.2).

In a study of <u>in situ</u> dog diaphragm length-tension curves, Kim et al (139) claimed that the diaphragm could generate active force at a length as short as 35%-40% of resting <u>in situ</u> length and concluded that the diaphragm works on a broader range of effective length than other skeletal muscles. McCully and Faulkner (176) studied the length-tension relationship of the diaphragm from five species and showed that the <u>in vitro</u> diaphragmatic length-tension relationship was similar to that of the limb muscles. This was subsequently supported by similar measurements made on the <u>in vivo</u> dog diaphragm (219) and <u>in vitro</u> rat diaphragmatic strips (102).

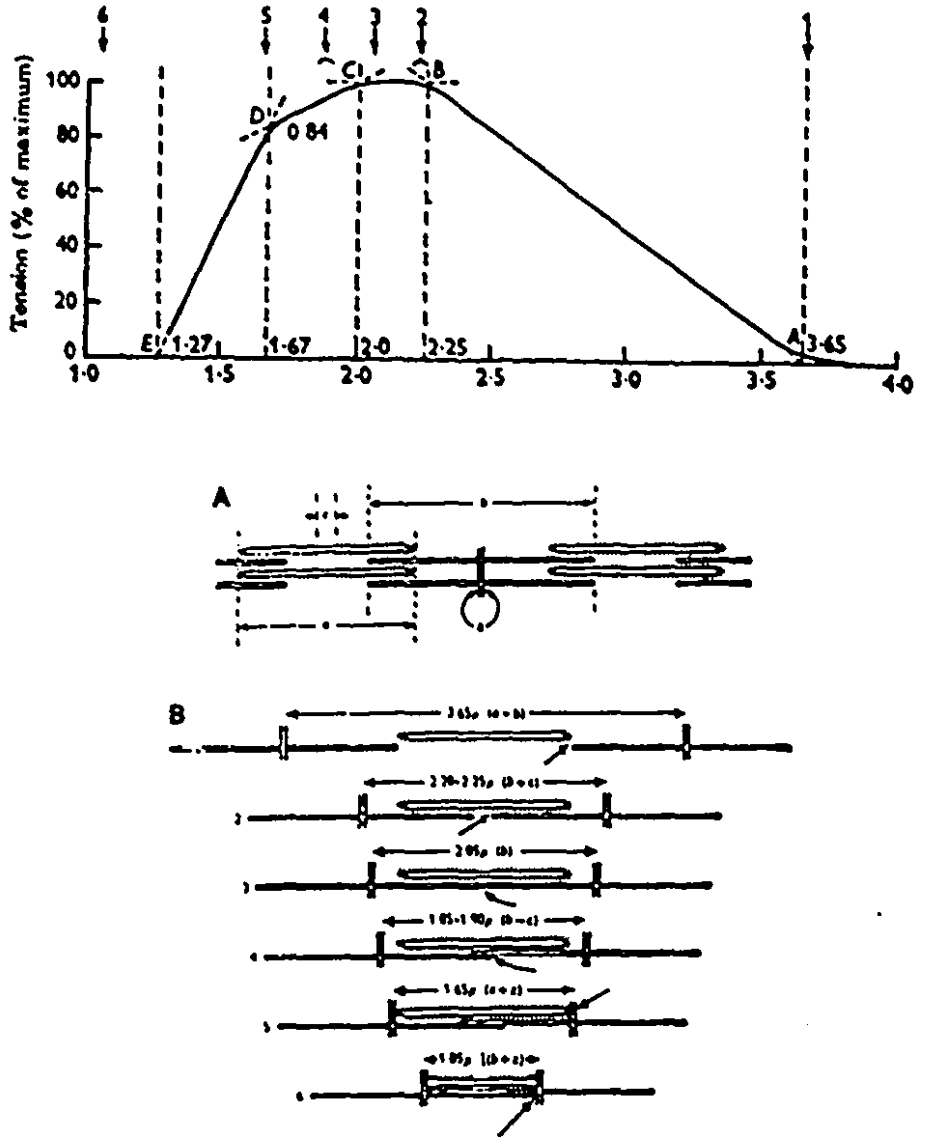

Figure 1.2: Active length-tension curve of frog muscle in relation to sarcomere length and degree of overlap between thin and thick filaments [adaped from Gordon et al (109)].

These authors attributed Kim's findings to the method used to determine the resting length which could underestimate the optimal length by 20%, and the longer duration of stimulation employed.

In the in vivo animals (139,209,232) and humans (5,121,209,238), the pressure exerted by the diaphragm varies inversely with increasing lung volume. This has been explained on the basis of the length-tension relationship. Indeed, it was demonstrated in normal subjects that the maximal voluntary inspiratory pressure and Pdi were inversely related to diaphragm length measured with chest X rays (32). It can be immediately seen that the in vivo diaphragm length must also be inversely dependent on lung volume provided that diaphragmatic shortening has produced the increase in volume (103,104). Optimal length is thought to be near residual volume (RV) and the length approximately 35% shorter at relaxed total lung capacity (TLC) (32,139,161,205,238). However, interpretation of in vivo data must take into consideration factors other than lung volume. These include diaphragm shape (170) and chest wall configuration (113), which presumably influence Pdi developed for any given diaphragmatic force development. Force and pressure are not necessarily tightly linked. Marshall (170) proposed that with increasing lung volume the diaphragm not only shortens but also changes its shape to a larger radius of curvature, which would also decrease the ability of the diaphragm to generate pressure for a given force according to the Laplace law. Whether or not the diaphragm follows the Laplace law is however controversial. Many investigators think it does not apply.

Kim et al (139) compared Pdi with the active tension produced by phrenic nerve stimulation as measured by a strain gauge in dogs over different lung volumes and found a close correlation (r=0.998) between these two parameters. Studies on humans also showed that there was no appreciable two dimensional shape change of the diaphragm between RV and TLC (32,103,161) and the dependence of Pdi on lung volume could be solely explained by the length-tension relationship of the diaphragm (238). These studies suggest that within the physiological range of lung volume shape change of the diaphragm is probably not big enough to influence diaphragmatic pressure generation significantly. However, there is little information on the three dimensional diaphragm shape change nor on force-length properties of the diaphragm orthagonal to the fibre direction during relaxed and contracted conditions. This information is essential if one wishes to apply the Laplace law to relate diaphragm force development to Pdi.

As has been suggested by Grassino et al (113), diaphragm fibre length may change at a given lung volume as a result of different chest wall configuration and therefore generate different force. In particular, abdominal configuration is an important determinant of the diaphragm length (70,140). However, during relaxation, there is a unique chest wall configuration at different lung volumes (142). As configuration determines fibre length at a given lung volume it is thus probable that only when contraction is elicited during relaxation is the relationship between the pressure developed by the diaphragm and lung volume explained to a large extent by the

length-tension relationship.

For the extra-diaphragmatic inspiratory muscles, the length-tension relationship has been studied in dogs. Farkas et al (88) demonstrated that with shortening below optimal length, parasternal intercostal muscle generates less force relative to its maximal force compared to the diaphragm. In another words, the descending limb of the length-tension relationship below optimal length is steeper in parasternal intercostal muscle than in the diaphragm. However, in supine dogs, parasternal intercostal muscle only shortens 10% between RV and TLC (68). In addition, unlike the diaphragm with a length at functional residual capacity (FRC) probably below its optimal length, the length of parasternal intercostal muscle at FRC is above its optimal length (88). One may thus expect that when lung volume undergoes a change from FRC to TLC, the length of the parasternal intercostal muscle must progressively shorten towards its optimal length, at least in supine dogs (88). Because of the significant difference of the mechanical arrangement between the diaphragm and parasternal intercostal muscles, the latter should improve its mechanical effectiveness as an inspiratory pressure generator with increasing lung and rib cage volume in contrast to the diaphragm. This has recently been reported by Jiang and associates in dogs (129).

There are only limited studies on the effect of fatigue on the length-tension relationship of the respiratory muscles. In a <u>in vitro</u> rat diaphragm strip preparation fatigued at optimal length by repetitive stimulation, Gauthier et al (102) showed a greater

magnitude of decrease in force at shorter than at longer length. This was attributed to the possibility of action potential propagation failure along the T-tubular systems at short but not at long lengths (246). Similar results were also obtained by Farkas and Roussos (93) with the diaphragm fatigued at different length but the initial force generated at the beginning of the fatigue protocol was made equal by manipulating the stimulation frequency. There has been no equivalent data in humans in vivo. However, respiratory muscle endurance is reduced at lung volumes above FRC maintained during inspiratory resistive loaded breathing either actively with inspiratory muscle contraction (225) or passively with a positive end-expiratory pressure applied at the mouth (250). Thus with loading or decreased energy supply a faster rate of decay of respiratory muscle force at high lung volumes can be predicted. This may have clinical relevance for patients with chronic hyperinflation. These patient may be at risk of respiratory muscle fatigue not only because the respiratory muscles work at a mechanical disadvantage due to their length-tension relationship but also because the endurance of their respiratory muscles may be reduced. Furthermore, there may be a change in the mechanical linkage between the costal and crural parts of the diaphragm with hyperinflation, which impairs the ability of the ventilatory pump to handle loads (69,164,263).

Force-velocity relationships of skeletal muscle are illustrated schematically in figure 1.3 and described by a hyperbolic curve (119), which indicates that when a muscle shortens

against a load, the shortening speed and the force developed are inversely and curvilinearly related. By extrapolating this relationship to zero load and zero velocity, the maximal shortening speed and maximal force can be determined. The maximal shortening speed of a muscle is in turn dependent on the myosin ATPase activity of its fibers. It has been reported that the contraction time (232) as well as the maximal shortening speed (94,169,184,218) of the diaphragm, like its fiber type composition, is in between a fast and a slow muscle.

Shortening capacity is very important for respiratory muscle function as a ventilatory pump. Diaphragmatic fatigue not only affects force generation but also leads to significantly decreased amount and velocity of muscle shortening both in vitro (169) and in vivo (220). In particular, recovery of force, the amount of shortening, and the velocity of shortening at low stimulation frequencies is delayed in diaphragmatic fatigue and takes longer than three hours (220). Apparently low frequency fatigue of the diaphragm also significantly influences shortening capacity. However, as discussed above, the ability to generate force and the ability to shorten are not always equally affected by muscle fatigue. As shown by Mardini and McCarter (169) in their in vitro rat diaphragmatic strip model, force and shortening were affected to the same extent only when the diaphragm performed isometric contractions of long duration or when the muscle shortened against a small load.

In an in situ dog preparation, diaphragmatic muscle fiber

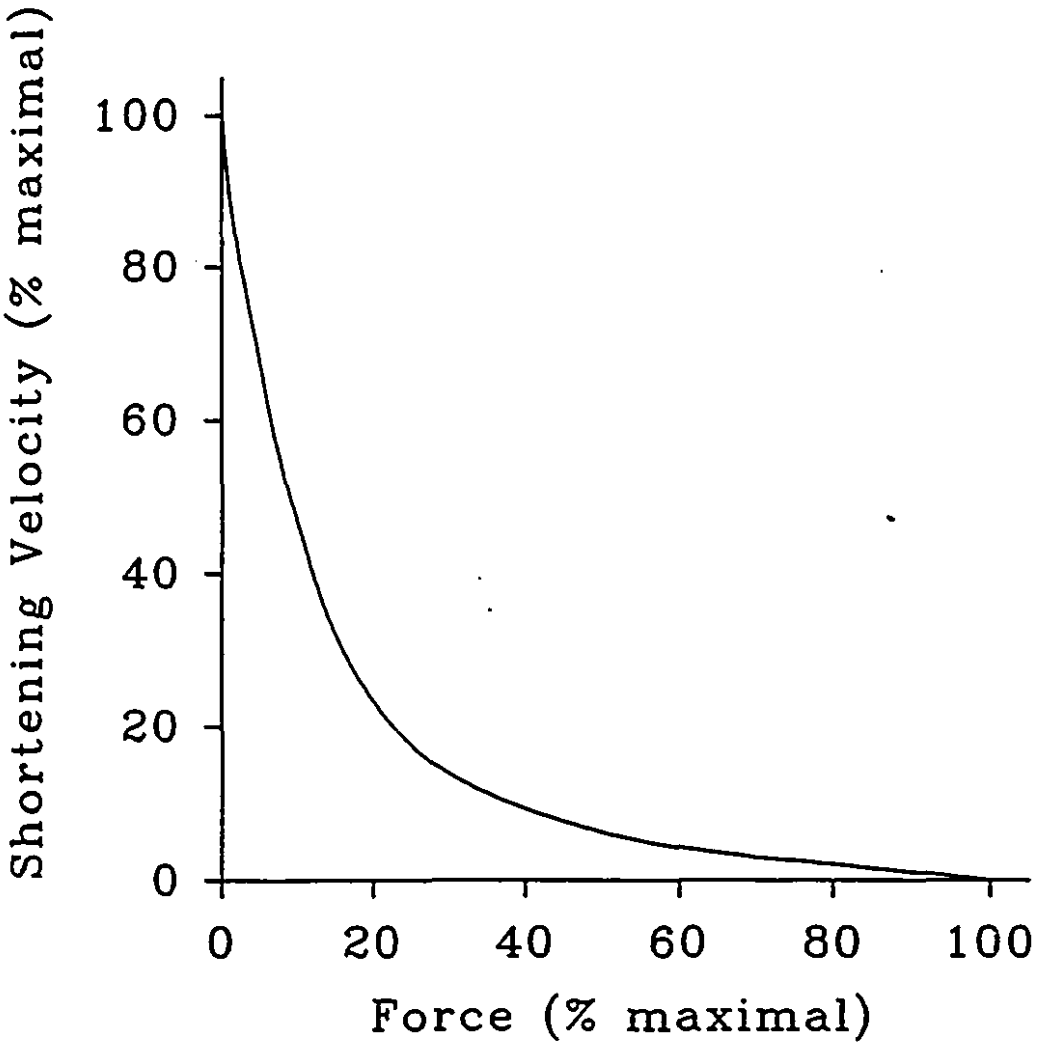

Figure 1.3: Schematic force-velocity relationship of skeletal muscle. Force and shortening velocity are both expressed as % of maximum.

shortening was measured by sonomicrometry and related to inspiratory flow (98,205). Although there was no unique relationship between instantaneous muscle shortening velocity and instantaneous flow within a breath (205), the mean velocity of diaphragmatic shortening has been shown to be linearly related to the mean inspiratory flow (98). Since force-velocity relationship of human respiratory muscles can not be directly evaluated in vivo, the pressure-flow relationship has been determined with voluntary inspiratory efforts (3,127,174) or diaphragmatic contraction elicited by phrenic nerve stimulation (209), in which inspiratory pressure change was taken as an index of force and inspiratory flow an index of shortening velocity. All these studies suggest a linear relationship between inspiratory muscle force and inspiratory flow. The fact that a linear relationship between pressure and flow is not in agreement with a hyperbolic relationship between force and velocity as shown by Hill for skeletal muscles (119) may at least in part be attributed to the added inspiratory resistance during the measurements of the above in vivo human studies (3,127,209), to the extent that some respiratory muscles still shorten considerably with a finite velocity with zero flow at the mouth during airway occlusion (98,205). With regard to fatigue, it has been shown in normal humans that increasing inspiratory flow rate shortens respiratory muscle endurance or Tlim (44,174) as is predictable from the hyperbolic shape of force-velocity relationship and the increased oxygen consumption in a shortened muscle (119). How the force-velocity relationship and its analogous pressure-flow

relationship of the respiratory muscles are affected by fatigue needs to be further investigated.

1.1.6 Respiratory muscle fatigue in patients

In respiratory disease, when the load on the respiratory muscles is increased, the strength decreased, the energy supplies impaired or the mechanical advantage diminished, it has been suggested that fatigue may result leading to ventilatory failure. This notion, however, depends largely on theoretical reasoning and on experimental results obtained from animals and normal subjects in whom fatigue has been induced during experiments in a laboratory environment. To what extent these results can be applied to patients remains uncertain and evidence of respiratory muscle fatigue in patients with ventilatory failure remains indirect and insufficient.

It has frequently been shown that PI,max (23,37,168,191,222,223,236) and Pdi,max (23,105,148) are low in patients with chronic air flow limitation compared with values from normal subjects. These measurements are even lower in hypercapnic than in non-hypercapnic patients (16,222). The decreased voluntary pressure generation by respiratory muscles in these patients could be largely a result of the mechanical disadvantage of their respiratory muscles rather than fatigue. In this connection, Similowski and coworkers (237) assessed diaphragmatic contractility in a group of stable COPD patients using bilateral phrenic nerve stimulation and compared the results with those of age-matched normal subjects. It was shown that at end-expiratory lung volume

the amplitude of diaphragmatic twitches was smaller in patients than in normals. The difference, however, could be entirely accounted for by hyperinflation and decreased diaphragmatic fibre length in the patients. In fact, when compared at the same % of predicted TLC, the Pdi twitch amplitude was greater in patients than in normal subjects (237). Well-preserved diaphragmatic contractility in stable COPD patients, as shown by Similowski et al (237), is not surprising. Studies on limb skeletal muscles have shown that chronic shortening of the muscle (~4 weeks) by immobilization with a plaster cast leads to a reduction in the number of sarcomeres in series whereas lengthening of the muscle results in additional sarcomeres being added. These changes reverse when the muscle is allowed to return to its optimal length (108,244,259). Such an adaptation of the skeletal muscles to chronic resting length change readjusts its individual sarcomere length so that the maximal tension can be developed in the shortened or lengthened state (260). Similarly, Farkas and Roussos (90) and Supinski and Kelsen (242) demonstrated <u>in vitro</u> that the diaphragm adapted to chronic shortening by shifting its length-tension curve in such a way that the maximal tension is minimally changed but generated at shorter length. A similar phenomenon was found in <u>in situ</u> animal preparations with chronic hyperinflation which demonstrate the same Pdi as control but developed at higher lung volumes during spontaneous breathing or following phrenic nerve stimulation (207). It seems likely that the diaphragm adapts to chronic hyperinflation by loss of sarcomere number (91,136).

Such an adaptation of the diaphragm is presumably present in COPD patients but may not completely compensate for the greatly shortened diaphragm because most of the patients showed a decreased $P_{I,max}$ or $P_{di,max}$.

It is surprising, however, that the ability to transform diaphragmatic force into a useful inspiratory fall in pleural pressure (as reflected by twitch $\Delta Pes/\Delta Pdi$ ratio) was shown to be greater in COPD patients than in normal controls no matter whether the comparison was made at FRC or TLC or at equivalent % of predicted TLC (237). Since diaphragmatic contraction produces negative pleural inspiratory pressure by descent of the dome and by outward displacement of the lower rib cage through insertional and appositional effects and both insertional and appositional effects become progressively less significant or even vanish with increasing lung volume (159,164), one would predict that the diaphragm from patients with chronic hyperinflation should have had impaired rather than preserved or even enhanced inspiratory function.

As pointed out by Macklem et al (163) the ratio $\Delta Ppl/\Delta Pab$ depends upon the mechanical linkage of the respiratory muscles and the relative compliances of the rib cage, abdomen and lung. Smith and Bellemare (238) assumed that Ppl was the driving pressure for the rib cage, whereas Pab drives the abdominal wall. Although the latter assumption is probably correct, the former is certainly wrong. One of the most complicated problems in the mechanics of breathing is the quantification of the pressure driving the rib

cage. It is not simply Ppl but a combination of Pab and Ppl. More recently, Chihara and Macklem (43) have shown that -ΔPpl/ΔPab during pure diaphragmatic contraction is determined by the ratio of abdominal compliance to the effective compliance of the rib cage (C'rc) where C'rc is the slope of the relationship between rib cage volume and Ppl during isolated diaphragmatic contraction. C'rc is considerably less than rib cage compliance measured during relaxation due to the rib cage distortion that occurs during pure diaphragmatic contraction and the fact that Ppl only acts on a portion of the rib cage. Their studies may suggest that a reduced rib cage distortability could account for the well preserved ΔPpl/ΔPdi ratio in COPD.

The above evidence suggests that stable COPD patients probably do not have respiratory muscle fatigue during everyday life. Respiratory muscle contractility of this group of patients is probably well preserved and much better than expected considering the shortening of the respiratory muscles that occurs. However, the functional reserve of the respiratory muscles in these patients is reduced as the result of the increase in the inspiratory pressure swing per breath due to high airway resistance and the decrease in $P_{I,max}$ due to muscle shortening (16,23). Bellemare and Grassino (23) demonstrated that during quiet breathing, the TTdi of stable COPD patients is three times greater than normal subjects, the TTdi can be increased by 8 times in normals but only by 3 times in patients before reaching the fatigue threshold. This leads, as has been proposed (23), to a much greater likelihood for the

respiratory muscles of COPD patients to become fatigued. However, it appears that even though many COPD patients with hypercapnia are capable of augmenting alveolar ventilation and eliminating CO_2, they do not do so in order to avoid fatiguing their respiratory muscles (23,208). In another words, these patients choose to hypoventilate and retain CO_2 rather than to develop respiratory muscle fatigue (16). Similarly, animals breathing spontaneously against very high inspiratory resistive loads which lead to acute hypercapnia usually do not develop respiratory muscle fatigue (2,95). These results lead to a hypothesis that respiratory muscle fatigue does not contribute to chronic hypercapnia in COPD patients whereas respiratory muscle weakness certainly does (221).

Even less information is currently available for respiratory muscle function and fatigue in critically ill patients. Most of our present knowledge on this topic is still based on the study done by Cohen and associates (46). These authors demonstrated that 7 out of 12 patients with difficulty weaning from mechanical ventilation showed EMG signs of diaphragmatic fatigue during a weaning trial, and 6 out of these 7 patients eventually developed hypercapnia. During weaning these patients exhibited the following sequence of events: firstly EMG signs of diaphragmatic fatigue appeared, this was followed by an increase in breathing frequency, which was, in turn followed by abnormal chest wall motion referred to as "respiratory alternans and abdominal paradox", finally in some patients the weaning attempt ended with a fall in minute ventilation and breathing frequency with resultant CO_2 retention

and acute respiratory acidosis. Weaning failure of these patients was attributed to development of inspiratory muscle fatigue and the abnormal chest wall displacements were proposed as clinical signs of inspiratory muscle fatigue. Similar results and conclusions were also reported subsequently by Pourriat et al (212) who demonstrated that during a weaning trial the patients with weaning difficulty generally had a low Pdi,max and high Pdi/Pdi,max ratio with abnormal chest wall motion whereas the patients with successful weaning did not. That inspiratory muscle fatigue may be a cause of weaning failure was also supported by findings of Brochard et al (34) who showed that inspiratory pressure support during a weaning trial could prevent EMG signs of diaphragmatic fatigue and suppress the sternomastoid muscle EMG activity that otherwise occurred. On the other hand, Tobin and colleagues (249) proposed that the low tidal volume and high respiratory frequency could explain up to 81% of hypercapnia developed in patients during a failed weaning trial, and that the rapid shallow breathing pattern occurred instantaneously when the patients switched from mechanical ventilation to spontaneous breathing. The authors reasoned that this rapid change of breathing pattern was largely responsible for the development of CO_2 retention and that weaning failure must have reflected a resetting of the brainstem respiratory centres in response to the suddenly increased load and could not be explained by respiratory muscle fatigue which should have developed more gradually. This hypothesis, while attractive, could not explain Cohen et al's observation that the initial development of rapid

shallow breathing was accompanied by a decrease rather than an increase in $PaCO_2$. By quantitative analysis of chest wall motion during weaning attempts, Tobin et al (247) also suggested that abnormal chest wall motion seems to be a common manifestation in the early stages of weaning from mechanical ventilation. They suggested that its presence possibly reflects increased respiratory load and does not necessarily predict weaning outcome. A similar analysis was also performed in normal subjects which demonstrated a close association between the degree of abnormal chest wall motion and inspiratory load and a dissociation between the abnormal chest wall motion and diaphragmatic fatigue (248).

Unfortunately, all these studies were based on procedures which assessed respiratory muscle function and fatigue indirectly. With the available information, we are still unable to state confidently whether or not respiratory muscle fatigue exists in patients, especially in those who are critically ill. In another words, we do not yet know if respiratory muscle fatigue is a cause of ventilatory failure or not.

1.2 Diagnostic procedures of respiratory muscle fatigue.

1.2.1 EMG power spectrum analysis.

It has been well established that fatiguing contractions of skeletal muscles are associated with a progressive decrease in muscle's EMG power spectrum from higher to lower frequencies (153). This change has been most frequently attributed to slowing of the conduction velocity of myoelectrical activity along the muscle fibre membrane during fatigue (22,154,181,192), possibly as a consequence of fatigue-induced metabolite accumulation in muscle fibres (192). However, there has been no agreement on the precise underlying mechanisms of the muscle power spectral shift during fatigue. For instance, Merletti et al (181) showed that in exercising muscle leading to fatigue, the average initial rate of decrease in conduction velocity was only 31%-72% of that of the EMG frequency shift. Bigland-Ritchie et al (25) failed to demonstrate any relationship between the change in conduction velocity and the power spectrum during fatiguing muscular contraction. These authors claimed that with fatigue the changes in EMG power spectrum may also be affected by factors other than the slowing of conduction velocity, possibly including the synchronization in the firing of motor units, the reduction in the motoneuron firing frequency, and a functional impairment of neuromuscular junction (111).

In spite of the many debates about the underlying mechanisms responsible for the shift of muscle EMG power spectrum to lower frequencies with fatigue, extensive work has been done to use this technique to identify the presence of muscle fatigue and three

parameters have been worked out to describe the frequency distribution of myoelectrical signals: EMG high-to-low frequency ratio (H/L), EMG mean frequency (153), and EMG median frequency or centroid frequency (234). All these parameters progressively decrease with fatigue although their changes may not be always of the same magnitude.

In 1979, Gross and coworkers (116) began to use EMG power spectral analysis to evaluate fatigue of respiratory muscles. These author showed that diaphragmatic EMG H/L was independent of Pdi when the diaphragm remains fresh but progressively decreased with Pdi generation known to produce fatigue (50% and 75% Pdi,max). Bellemare and Grassino (22) demonstrated that the rate of decay of diaphragmatic EMG H/L characterized by its time constant (T_F) was linearly and inversely related to TTdi. Faster decay occurred with higher TTdi and T_F was infinite when TTdi was smaller than 0.15 below the fatiguing threshold (21). Since then, EMG power spectral analysis has been extensively employed to confirm the presence or absence of respiratory muscle fatigue both in normal subjects (11,15,30,31,36,85,97,100,123,133,195,243,254) and in patients (23,34,46,110,141,149).

It has been shown that the shift of diaphragmatic EMG power spectrum to lower frequencies occurs in the first few breaths against a load with TTdi greater than 0.15 at a time when the target pressure is well maintained (22,116). Upon cessation of the fatiguing task the change quickly returns to control level (22,195). Therefore, the power spectral shift is a sign signifying

that the muscle is becoming fatigued and occurs before task failure. This characteristic makes it possible for this technique to predict fatigue and task failure. On the other hand, its quick recovery within a few minutes after load removal limits its value to detect only high frequency fatigue. It does not reflect low frequency fatigue, which generally lasts for many hours and is presumably the most clinically important type of respiratory muscle fatigue in patients. Furthermore, there is only a very short time frame to detect the spectral shift, which limits it diagnostic usefulness. Indeed, Moxham and associates (195) found that the decline and recovery of H/L closely followed those of high frequency fatigue but failed to detect the persistent presence of low frequency fatigue of quadriceps and the diaphragm. This indicates that the shift in the power spectrum does not reflect the reduced ability for the muscle to develop either force or velocity of shortening, which are the important changes in muscle fatigue (217). Another potential problem of this technique is its vulnerability to interference from cardiac artifacts (22,116,234). It is conceivable that the amplitude of the ECG with its substantial low frequency power will by itself be interpreted as a power spectral shift of the EMG, particularly with tachycadia. If the ECG contaminates the EMG a power spectral shift could be interpreted as a change in the physiological properties of the diaphragm which did not occur al all. Under this situation, appropriate gating techniques that remove ECG but recover EMG signals are required. This is certainly inconvenient for bed-side

monitoring of respiratory muscle function in patients. Finally, the power spectrum parameters are not reproducible for a given individual on separate occasions (116), so that each test has to serve as its own control during an on line measurement; that is, a patient has to be tested during both fatigued and unfatigued conditions. Because of these limitations, power spectral analysis, although widely used in research laboratories to assess fatigue, is not suitable as a clinical diagnostic test for detection of respiratory muscle fatigue in patients.

1.2.2 Rate of relaxation:

The relaxation of skeletal muscles from a previous isometric contraction has a well-defined time course and the rate of relaxation slows when the muscle is fatigued (78,130). The proposed mechanisms responsible for the slowing of relaxation during fatigue include a reduced rate of calcium reuptake into sarcoplasmic reticulum (33) and a reduced rate of cross-bridge detachment (78,79), the latter may in turn depend on the muscle metabolic abnormalities seen during fatigue (38,79,80).

Two parameters have been used to measure relaxation rate: [1] the maximal relaxation rate (MRR) which is the slope of the tangent at the steepest point of muscle force decrement during relaxation. This is usually normalized by the amplitude of the force; [2] the time constant (τ) of the monoexponential force decay during the later 50%-60% of relaxation. Both parameters have been used to evaluate fatigue of respiratory muscles.

Esau et al (85) studied the changes in MRR and τ from Pdi

tracing when the subjects performed voluntary inspiratory efforts against inspiratory resistance with different TTdi and found that there was a progressive decrease in MRR and increase in τ with increasing TTdi above 0.20 and a constant MRR and τ with TTdi below 0.20. In addition, there was a close linear relationship ($r \geq 0.90$) between Pdi relaxation rate parameters and diaphragmatic EMG H/L. Subsequently, studies using stimulated diaphragmatic contraction added the evidence that the relaxation rate of the diaphragm decreases when the muscle is fatigued (13,86). These studies suggest that relaxation rate is potentially useful to evaluate respiratory muscle fatigue. Furthermore, it was claimed that relaxation rate measured during a sniff closely resembles that measured during a voluntary (86,150) or stimulated (86,257) diaphragmatic contraction. Because Pdi and Pm have the same relaxation rate (144,150), either can be used to assess respiratory muscle fatigue. This raises the possibility that fatigue might be diagnosed by measuring the relaxation rate non-invasively.

However, observations made on COPD patients demonstrated that relaxation rates measured from mouth pressure tracings could not be used to evaluate respiratory muscle fatigue in this group because the time constant for equilibration of pressure between alveoli and the mouth was too long, leading to a misinterpretation of the patient's true relaxation rate (200). These investigators (202) also showed that respiratory muscle relaxation rate measured during unoccluded sniffs was progressively increased with increasing amplitude of the sniff even when normalized by peak sniff pressure

unless the peak sniff pressure was over 60% of the control maximal. This could be explained either by the progressive recruitment of fast twitch fibres with increasing voluntary efforts leading to faster relaxation as originally described by Wiles et al (258) for the quadriceps or by the fact that greater unoccluded sniffs generated greater lung volume changes and therefore caused greater respiratory muscle shortening, which, as has been suggested (93,150,238), accelerated the relaxation. These studies indicate that the proper interpretation of the results depends upon measurement of relaxation rate of Pdi or Pes tracings with involuntary respiratory muscle contractions. However, this is not so simple and non-invasive as originally proposed compared with other available techniques to evaluate respiratory muscle fatigue.

More importantly, the diminished muscle relaxation rate as a result of fatigue quickly returns to control level with a time course similar to the EMG power spectrum shortly after the elimination of the fatiguing load (86,122,144,201), does not follow the time course of low frequency fatigue, and therefore does not detect the persistant decrease in contractility that occurs. This feature casts doubt on the suitability of relaxation rate as a useful technique to assess respiratory muscle fatigue in patients.

1.2.3 Endurance test:

For a given tension time index, the endurance properties of the respiratory muscles can be evaluated by comparing the length of Tlim or the rate of decrease in force. Respiratory muscle endurance properties have been studied in animals (93,128,216,235,253), in

normal subjects (21,42,44,73,82,96,155,174,175,225,226,250), and in patients (49,83,141,177,191,204,208).

Many factors may influence respiratory muscle endurance. In vitro studies showed a faster decrease in respiratory muscle force in isotonic than isometric contractions (235). Similarly, in vivo work demonstrated a shortened respiratory muscle endurance at high lung volumes compared with lower volumes (225,250). Hypoxia may impair respiratory muscle endurance properties (226) especially during isotonic contractions (10). It has also been shown that high tidal volume (44) and high flow rate (44,45,73,174,175) decrease respiratory muscle endurance. All these factors have to be taken into consideration to evaluate endurance.

Endurance tests can not be used as a routine to assess respiratory muscle function and fatigue in patients. It is risky and unacceptable in severely-ill patients because recovery is uncertain although such tests have been done on stable patients without apparent ill effects. Any test based upon inducing fatigue to assess endurance runs the risk that whenever the test is over fatigue will persist, because the respiratory muscles do not obtain sufficient rest to recover. In addition, respiratory muscle endurance is highly variable among different individuals dependent not only on physical fitness but also on motivation of the subject. Comparison must be made at equal values of tension time index and this requires a maximal voluntary effort and appropriate scaling of the load that are frequently not possible in sick patients. A further complication is that increased expiratory muscle activity

and decreased end-expiratory lung volume have been found during inspiratory resistive loading both in animals (173) and in humans (1,126,137,171,180). This may also contribute to the variation of inspiratory muscle endurance since decreased FRC assists diaphragmatic function and therefore may delay fatigue.

1.2.4 Voluntary forces

The strength of the respiratory muscles can be assessed by measuring $P_{I,max}$ (29,47,230,231,261) and $P_{di,max}$ (5,226) generated during a static voluntary efforts. A low value of $P_{I,max}$ (23,37,168,191,222,223,236) and $P_{di,max}$ (23,105,148) has frequently encountered in patients with chronic airflow limitation suggesting respiratory muscle weakness.

$P_{I,max}$: This measurement is generally done by recording the pressure change in the mouth with the subject performing a maximal inspiratory Mueller manoeuvre against an airway occlusion with a small leak introduced to keep the glottis open (29). A decrease in the capacity to generate $P_{I,max}$ at a given lung volume over time presumably reflects inspiratory muscle fatigue (225). The development of $P_{I,max}$ involves the co-activation of the diaphragm and rib cage inspiratory muscles. It is still uncertain whether or not the respiratory muscles can be maximally activated during a maximal inspiratory Mueller manoeuvre. Gandevia and McKenzie (101) found that normal subjects could maximally activate their diaphragm during a maximal inspiratory Mueller manoeuvre but Hershenson and colleagues (118) showed that the diaphragmatic activation during such a manoeuvre was always submaximal. In addition, it is not

possible with present technology to evaluate rib cage muscle activation. Furthermore and most importantly, it is not known whether or not sick patients are able to maximally activate their respiratory muscles. One must be cautious in interpreting $P_{I,max}$ data because the "learning effect" or "warming-up effect" are obvious in some untrained subjects. Recently, Multz et al (199) demonstrated that in sick patients with mechanical ventilation, $P_{I,max}$ is highly variable for the same patient among days (average CV: 28.5%) or on the same day among different investigators (average CV: 32%) even though the triplicate tests done in a single sitting by a single investigator showed high reproducibility. These authors (199) further pointed out that even their "cooperative patients" were not cooperative enough to give maximal inspiratory efforts and the overall $P_{I,max}$ thus obtained on different occasions by different staff frequently reflects submaximal inspiratory efforts. The measurement thus tends to underestimate inspiratory muscle strength. In another words, $P_{I,max}$ measurement in severely-ill patients is far less reliable than previously thought.

$P_{di,max}$: The concept of using Pdi to reflect the force generated by the diaphragm in vivo was put forward in 1960s by Agostoni and Rahn (5) and validated in dogs by comparing the Pdi and simultaneously and directly recorded diaphragmatic tension by Kim et al (139). In humans, Pdi,max is generally determined by subjects performing an inspiratory Mueller manoeuvre or a Mueller manoeuvre plus an abdominal expulsive manoeuvre. There is a great variability of normal values for Pdi,max reported by different

authors as listed in table 1.1. It can be seen that the Pdi,max determined with Mueller plus expulsive manoeuvre is significantly greater than that with inspiratory Mueller manoeuvre but the variability is high even within a given manoeuvre.

Table 1.1: Maximal transdiaphragmatic pressure [adapted from Laporta and Grassino (147)].

Author (Ref.)	Sub.	Transdiaphragmatic pressure (cmH_2O)	
		Mueller	Mueller+Exp.
Agostoni (5)	4	97	
Milic-Emili (187)	6	138±22	
Roussos (226)	3		217±55
Moisan (190)	8	141	
Gibson (105)	11	126±45	165±57
Aubier (11)	4		192±17
De Troyer (60)	10	99±40	116±8
Bellemare (21)	4		190±30
Braun (32)	7	114±19	
Miller (188)	44	97±35	

Several factors in addition to subjects' physical characteristics such as age and sex (42) are responsible for the great variation of Pdi,max measurement. Firstly, Pdi is dependent on diaphragmatic fibre length. This is determined by both lung volume and, at a given lung volume, on chest wall configuration. The chest wall can assume different shapes and therefore different

fibre lengths at a given lung volume (113). Secondly, during a maximal inspiratory Mueller manoeuvre, diaphragmatic activation could be submaximal (118). Thirdly, during combined Mueller and abdominal expulsive efforts, although the diaphragm may be maximally activated (17,118), the abdominal muscles may not be (118); the Pdi,max thus determined is dependent on the maximal achievable Pab which in turn depends on the magnitude of abdominal muscle activity. Furthermore, it was recently suggested that when abdominal expulsive efforts are superimposed on a Mueller manoeuvre, the additional Pdi generated is largely a result of mechanical stretching of the contracting diaphragm provided by abdominal muscles independent of diaphragmatic maximal force, this may lead to overestimation of diaphragmatic strength (120). For these reasons, it has been pointed out that accurate determination of Pdi,max requires a constant rib cage-abdominal configuration (60,113) and visual feedback of pleural and abdominal pressures to the subject (60,147). This, however, meets the condition cited by Hershenson and associates (118) for submaximal activation of the stronger muscle (presumably the diaphragm). One is forced to the conclusion that a valid measurement of Pdi,max while controlling diaphragmatic fibre length even in normal subjects is a futile exercise. In patients it has little clinical potential.

Inspiratory strength determined during maximal sniffs: A method to assess inspiratory muscle strength by recording Pes and Pdi during a brief and sharp unoccluded maximal sniff has been suggested by Mier et al (185) and Laroche et al (148). It was

claimed that this technique is simple, quickly learned, reproducible, and more sensitive than $P_{I,max}$ or diaphragmatic twitch to detect inspiratory muscle weakness (143,148,185,186). It was also suggested that nasopharyngeal pressure (Pnp) and mouth pressure (Pm) measured during a maximal sniff reflect Pes changes, therefore installation of an esophageal balloon is not necessary to assess inspiratory muscle function using the sniff technique (143). The drawback to this technique is the complete lack of knowledge about the extent of inspiratory muscle activation during a "maximal sniff". In addition, during a maximal unoccluded sniff, there is a substantial increase in lung volume at high flow rates leading to an inspiratory muscle contraction in which shortening velocity and length are uncontrolled. The role played by upper airways and its impact on inspiratory pressure generation during such a very dynamic manoeuvre are also poorly defined. Finally, it was recently demonstrated that Pnp or Pm could not be used to reflect Pes during the sniff manoeuvre in COPD patients because of the prolonged airway time constant (200).

All these measurements require maximal voluntary efforts and special respiratory manoeuvres and thus highly motivated and cooperative patients. These requirements are often too difficult for severely-ill patients who have no previous experience in performing the necessary manoeuvres. Assessment of respiratory muscle function in patients, therefore, needs an objective parameter which can be measured independently of the patient's voluntary efforts.

1.2.5 Phrenic nerve stimulation:

When the phrenic nerves are electrically stimulated, the resulting diaphragmatic action potentials and the mechanical response measured as Pdi change can be recorded. With supramaximal tetanic stimulation at different frequencies, Pdi-frequency curves of normal subjects have been obtained. This is similar to that of other skeletal muscles (19,196) and to shift to the right with diaphragmatic fatigue (11,19,196-198). This shift of Pdi-frequency relationship during fatigue can be expressed by a decreased ratio of Pdi at a lower over that at a higher frequency signifying low frequency diaphragmatic fatigue (11,195,196).

Two major problems limit the usefulness of tetanic phrenic nerve stimulation from becoming a diagnostic procedure for detection of diaphragmatic fatigue. Firstly, tetanic phrenic nerve stimulation is painful and hard to tolerate even by trained subjects. Secondly, bilateral phrenic nerve stimulation is necessary for assessment of diaphragmatic contractility when this is measured as the difference between gastric and esophageal pressure. For gastric pressure to truly represent the abdominal pressure developed by diaphragmatic contraction, activation of both hemidiaphragms simultaneously is required. The arithmetic sum of Pdi elicited by separate unilateral left and unilateral right stimulation is less than that resulting from bilateral stimulation (19,186), a phenomenon attributable to the distortion of abdominal contents and the diaphragm following unilateral stimulation with descent of one hemidiaphragm and elevation of the other (19).

However, maintenance of bilateral trains of supramaximal phrenic nerve stimulation is almost impossible due to the extreme discomfort and strong co-stimulation of neck muscles (19).

Bellemare and Bigland-Ritchie (17) introduced the twitch occlusion method to study diaphragmatic activation and contractility. This technique superimposes bilateral supramaximal phrenic nerve single shocks on ongoing diaphragmatic voluntary contraction. With increasing intensity of voluntary diaphragmatic contraction, the size of the elicited diaphragmatic twitches is progressively decreased until no twitch can be elicited indicating maximal diaphragmatic activation (17). With this technique, not only can the activation of the diaphragm be evaluated so as to detect central fatigue but also Pdi,max can be estimated during submaximal diaphragmatic contraction by extrapolation of the relationship between twitch and voluntary Pdi to zero twitch. The twitch occlusion method has now been successfully applied to evaluate diaphragmatic activation both in normal subjects (17,101,118,151) and in stable patients with chronic airflow limitation (177,204,237). Because twitch occlusion demands patients' cooperation, it remains questionable whether or not this technique can be applied to sick patients.

Diaphragmatic twitches in response to bilateral supramaximal phrenic nerve shocks have also been recorded in both normal subjects (13,17,19,101,118,121,151,186,237,238,257) and patients (12,186,204,237) during relaxation. There is a great variability of twitch amplitude among subjects and therefore a considerable

overlap of the values between normal subjects and patients and between the fresh and fatigued diaphragm. However, the diaphragmatic twitch obtained from a given subject was shown to be quite reproducible over time (13,17,186). Furthermore, diaphragmatic fatigue induced by inspiratory resistive loading consistently and significantly decreased the amplitude of twitch Pdi in all the subjects tested (13). These studies suggest that although a single determination has a limited value, serial determinations over time for a given patient provide valuable information about changes in diaphragm contractility. Therefore bilateral supramaximal phrenic nerve stimulation with single shocks has the potential to become a diagnostic test for detection of diaphragmatic fatigue in patients (217).

The major advantage of phrenic nerve stimulation over other existing techniques is that it provides a measure of diaphragmatic force that is independent of voluntary effort. The second advantage is that a decreased diaphragmatic twitch amplitude with delayed recovery following fatigue as shown by Aubier et al (13) is a direct indication of low frequency diaphragmatic fatigue. Low frequency fatigue is prolonged and lasts for hours, in contrast to high frequency fatigue from which recovery is less than one hour. Assessment of maximal pressures, EMG power spectral shift, and relaxation rate all reflects high frequency fatigue, whereas twitch Pdi in response to supramaximal stimulation reflects clinically relevant low frequency fatigue. Although needle electrodes have been used for phrenic nerve stimulation by many investigators

(12,13,118,121,257), transcutaneous stimulation (17,19,151,186,237,238) is quite practical and certainly more preferable for patients because of its non-invasive nature.

However, twitch Pdi in response to a fixed level of phrenic nerve stimulation can be modified by factors other than changes in diaphragm contractility. Firstly, binding of the abdomen significantly potentiates the twitch Pdi amplitude because of the decreased shortening and velocity of shortening of diaphragmatic fibres (17,257). This is evident because in vivo isolated diaphragmatic contraction causes outward displacement of the abdomen and inward displacement of the rib cage (121). Thus a decreased amplitude of twitch Pdi is explained by decreased diaphragm contractility only when length change and shortening velocity are controlled variables. Indeed, it has been shown that diaphragmatic twitch amplitude is inversely related to lung volume (121,238), a phenomenon largely explained on the basis of the force-length relationship of the diaphragm (32,139). This indicates that to be useful as a diagnostic test, diaphragmatic twitch has to be determined as a function of lung volume; however, the manner by which fatigue modifies the twitch Pdi-lung volume relationship has not been described. Finally, as suggested by Grassino et al (113), twitch Pdi amplitude will also be affected, at a given lung volume, by chest wall configuration as configuration determines fibre length. To solve this problem, twitch Pdi has to be measured during relaxation, a circumstance in which there is a unique chest wall configuration for a given lung volume (142) and therefore a unique

length. Respiratory muscle relaxation is a problem in the majority of untrained subjects but could be achieved in mechanically ventilated patients in whom respiratory muscle activity is largely suppressed.

Agreement was recently reached by respiratory muscle experts (217) that bilateral transcutaneous supramaximal phrenic nerve twitch stimulation has the best potential of becoming a diagnostic test for respiratory muscle fatigue. The measurement of mouth pressure against an occluded airway as an index of Pdi may be combined with phrenic nerve stimulation. This may eventually make the test even less invasive and therefore more applicable. More rigorous evaluations of this test, however, need to be done before it can be recommended for general practice.

1.3 Action, recruitment, and coordination of respiratory muscles

1.3.1. Action of respiratory muscles

Respiratory muscle action is a complex topic and has been the subject of controversy since at least the time of Hippocrates. The complexities arise from the geometry of respiratory muscles, their arrangement, and the complex structures on which respiratory muscles work. This section can only give a very brief summary.

The diaphragm: Contraction of the diaphragm creates a negative intrathoracic pressure swing which inflates the lung by descent of the diaphragm dome (161) and expansion of the lower part of the rib cage (159). The upper part of the rib cage inevitably moves inward during isolated diaphragmatic contraction both in animals (53) and humans (121), this can be directly related to the fall in pleural pressure during diaphragmatic contraction since elimination of the fall in pleural pressure can prevent the inward displacement of the upper rib cage (53). The lower part of the rib cage in humans moves outward during diaphragmatic contraction through two agencies, namely insertional and appositional effects of diaphragmatic contraction (159).

Both insertional and appositional effects depend on the zone where the diaphragm is apposed to the inner surface of the rib cage called the zone of apposition (178) and estimated to be about 40% of total rib cage surface area at FRC (179). In the zone of apposition, in dogs the diaphragmatic fibres run perpendicular to the costal margin from which they arise in a cephadodorsal direction; in humans they run axially and exert a cephalad force at

their points of origin on the costal margin thus expanding the lower ribs. This expansion is largely dependent on the impedance to caudal motion provided by abdominal viscera, abdominal muscles, and the abdominal wall which determine abdominal compliance (66). This effect of diaphragmatic contraction is called the insertional component of diaphragmatic contraction and is inflationary to the rib cage. The second agency though which the diaphragm acts on the lower rib cage is called the appositional component of diaphragmatic contraction. The increase in abdominal pressure during diaphragmatic contraction is transmitted through the zone of apposition to the lower rib cage. This is also inflationary and dependent on the size of the zone of apposition, the magnitude of the increase in abdominal pressure, and any change of abdominal pressure across the diaphragm (66,159,178).

Experiments on dogs (65,66) have shown that the contraction of the costal and crural parts of the diaphragm has qualitatively different effects on the lower rib cage. Contraction of the costal diaphragm inevitably expands the lower rib cage, an effect dependent on the resistance of abdominal contents and the magnitude of abdominal pressure development; contraction of the crural diaphragm does not have a direct effect because it lacks attachments to the rib cage. It has an indirect effect on lower rib cage dimensions dependent on the balance between the inflationary effect of rising abdominal pressure and the deflationary effect of falling pleural pressure (66). These results suggest that the inflationary action of the diaphragm on the lower rib cage through

the so-called insertional and appositional components of diaphragmatic contraction is mainly accomplished by the costal diaphragm and supports the notion that the diaphragm actually consists of two muscles.

In keeping with the concept that the diaphragm is composed of two muscles, Macklem et al (164) proposed a serial and parallel model of diaphragm action. These authors pointed out that since the costal and crural parts of the diaphragm have different actions on the rib cage (65,66) and the force developed by one part is not transmitted to the other part, the two parts must be arranged mechanically in parallel. Such a mechanically parallel arrangement predicts that the forces produced by the two parts are additive but the shortening or displacements are not. This model further predicts that with hyperinflation the costal and crural parts of the diaphragm are more and more arranged in series so that the forces of the two parts are no longer additive, indicating that the diaphragm is not well designed to handle large loads at high lung volumes (69,164,263).

The inspiratory rib cage muscles: The mechanical action of the rib cage muscles on rib cage displacement was unclear and has been a subject of controversy. In 18th century, based on his mechanical and geometric model analysis, Hamberger proposed that the external and internal intercostal muscles have an inspiratory and expiratory action, respectively, on the rib cage [see De Troyer and Loring (64)]. This notion is the basis of the conventional understanding of intercostal muscle action on the rib cage. In recent years,

direct muscle stimulation demonstrated a more detailed action of different rib cage muscles. De Troyer and Kelly (62) demonstrated that parasteral intercostal muscles are inspiratory to the rib cage. A subsequent study working in one to three interspaces (63) showed that isolated contraction of either internal or external intercostals has a similar effect on the rib cage primarily dependent on the resistance of the upper rib to caudal motion relative to that of the lower rib to cranial motion irrespective of muscle fibre orientations; at FRC, the net effect of intercostal muscle contraction is always a rib cage elevation resulting in expansion which is inverted to rib cage constriction at higher lung volumes for contraction of either internal or external intercostals. This observation was supported and further extended by Ninane et al (206) that the action of intercostal muscles is also critically dependent on their locations, to the extent that in the upper rib cage both internal and external intercostals are inspiratory at and above FRC, in the lower rib cage region both muscles have no inspiratory action at FRC and are expiratory at higher lung volumes. Using a finite-element analysis on dogs (162) and humans (158), Loring and Woodbridge demonstrated that when the forces of either external or internal intercostals were applied in a single interspaces, their effects were to bring the adjacent ribs close together. When the forces were applied to all intercostal spaces, external intercostals were inspiratory and internal intercostals expiratory to the rib cage, consistent with Hamberger's conventional notions. In addition, Whitelaw and

associates (256) demonstrated that the lateral intercostal muscles (both internal and external) also play an important role in producing axial rotations of the thorax.

1.3.2 Respiratory muscle use during quiet breathing

To accomplish quiet breathing in normal individuals requires generation of an inspiratory pressure that is only a very small fraction of the inspiratory muscles' maximal capacity. Grimby et al (115) and Goldman and Mead (107) proposed that the diaphragm was the only important contracting muscle during quiet breathing at least in upright humans. It was felt that the diaphragm contracting alone displaced both the rib cage and abdomen along their relaxation characteristics (107). These authors hypothesized that the diaphragm could accomplish this because abdominal pressure drove the relaxed rib cage along its relaxation configuration. Rib cage muscle activity during quiet inspiration was thought to be purely fixative (71).

However, it was subsequently observed that rib cage and abdominal motion during quiet breathing in quadriplegic patients with inactive rib cage muscles departed significantly from relaxation characteristics (55,193,233) and isolated diaphragmatic contraction with phrenic nerve stimulation induced paradoxical movement of the upper rib cage (121). If the diaphragm exerted an equal inflationary pressure on both the rib cage and abdomen, the diaphragm contracting alone should produce no chest wall displacement during a Meuller manoeuvre. However, it proved necessary to recruit the rib cage muscles in order to prevent

outward abdominal and inward rib cage movements during a diaphragmatic Meuller manoeuvre in humans (163). These studies indicated that rib cage inspiratory muscles are important agonistic muscles during quiet breathing to keep chest wall configuration close to its relaxation characteristic. It is now known that both parasternal intercostals (61,67,165) and scalenes (61) actively participate in quiet breathing and the activity of these muscles can not be easily suppressed even with very small tidal volume change in highly trained subjects. These observations suggest that the diaphragm, the inspiratory rib cage muscles, and even the accessory muscles are highly coordinated to perform the breathing task. Coordinated respiratory muscle contraction not only prevents chest wall distortion and maintain the minimal energy configuration but also optimizes respiratory muscle performance in terms of inspiratory pressure generation, as shown by DiMarco and associates (72) in dogs.

1.3.3 Respiratory muscle fatigue and ventilation

It was originally speculated that respiratory muscle fatigue would lead to ventilatory failure (225,226). Thus an important question to be answered is whether or not respiratory muscle fatigue does alter ventilation and its control. The available information suggests that respiratory muscle fatigue may induce decreased tidal volume and increased breathing frequency referred to as rapid shallow breathing.

During weaning trials in critically ill and mechanically ventilated patients, rapid shallow breathing with resultant

hypercapnia was frequently seen in weaning failure (46,212,249), accompanied by decreased Pdi,max and increased Pdi/Pdi,max ratio (212) or decreased diaphragmatic EMG H/L (46). Animal studies (2,229) showed that inspiratory resistive loaded breathing leading to respiratory muscle fatigue induced rapid shallow breathing and hypercapnic ventilatory failure. Rapid shallow breathing was also obtained in normal subjects breathing against inspiratory loads (180) and during exercise with a restricted rib cage (124). Because in these studies, direct evidence of respiratory muscle fatigue was not adequately provided, observations have been made in normal subjects to compare the breathing pattern before and after induced respiratory muscle fatigue. Following a global inspiratory muscle fatigue protocol in normal subjects, rapid shallow breathing became evident during spontaneous breathing (100). During exercise, inspiratory muscle fatigue mainly increases breathing frequency with a less pronounced decrease in tidal volume in normal subjects (166,167).

On one hand, rapid shallow breathing is not efficient for CO_2 elimination and the work of breathing performed against resistive loads. On the other hand, rapid shallow breathing has two major advantages in terms of respiratory muscle mechanics. Firstly, a smaller tidal volume requires a smaller intrathoracic pressure change during inspiration. As a result, the elastic work of inspiration diminishes and the tension-time index might diminish provided the relative pressure swings are sufficiently small. As a result, severe mechanical failure could be avoided or delayed

(21,22). Experimental evidence supporting this proposal came from observations in animals with inspiratory resistive loaded breathing. These studies showed that although rapid shallow breathing occurs early in the process, it is extremely difficult to induce respiratory muscle contractile failure (2,95,229). Secondly, a smaller tidal volume with a smaller intrathoracic pressure change may minimize inspiratory effort sensation (31,132,138,215), which may be increased after respiratory muscle fatigue (100,240).

1.3.4 Respiratory muscle use during augmented breathing

When ventilation is augmented such as during rebreathing or exercise, the activation of the diaphragm, the inspiratory rib cage muscles, and the expiratory muscles all increase. As a rule, the magnitude of increase in inspiratory rib cage muscle response is generally greater than that of the diaphragm.

In cats (54), rabbits (50,54), and dogs (51,52,251), CO_2 stimulated breathing induces an increase in rib cage inspiratory muscle activition that is proportionately greater than that of the diaphragm. In humans, no comparable data have been available. However, Pengelly et al (210), Chapman and Rebuck (40), and Chapman et al (39) have demonstrated that the tidal volume response to CO_2 is primarily determined by rib cage compartmental response and the rib cage response slope is significantly greater than abdomen. Similar results have been reported for hypoxic (39,41) and exercise response (39). In addition, Bye and coworkers (36) demonstrated a progressively increased slope of transpulmonary pressure-gastric pressure relationship [Macklem diagram (163)] during inspiration

with increasing ventilation during exercise. All these studies indicate that inspiratory rib cage muscles are preferentially recruited compared with the diaphragm with increasing ventilation. As a result of rib cage muscle recruitment, abdominal pressure swings diminish (163) and more of the pressure developed by the diaphragm is converted into a useful fall in Ppl.

Inspiratory rib cage muscles are also important in load compensation and posture response. During mechanical loading, both EMG response (9,52,117) and chest wall compartmental response (180) indicate a greatly augmented response of rib cage muscles over that of the diaphragm. Changing posture from supine to upright is associated with an increased phasic inspiratory EMG activity of the rib cage muscles in quiet breathing (74). During CO_2 rebreathing, altering posture has a greater effect on increasing rib cage muscle EMG activity than on diaphragm (262) and induces a greater rib cage compartmental response than abdomen (40).

The question arises: "Why does the inspiratory rib cage muscle contribution become progressively more important with increasing ventilation or load compensation?" Firstly, the diaphragm contraction per se produces chest wall distortion (53). Chest wall distortion is greater with higher ventilation and greater load (4). An augmented inspiratory rib cage muscle response may minimize chest wall distortion. Secondly, as has been recognized, rib cage muscles, unlike the diaphragm, possess plenty of muscle spindles (48), such that they are more suitable to detect and respond to changes in load and posture. Thirdly, as shown by D'Angelo and

Bellemare (51), when contracting alone, the relationship between tidal volume and EMG activity of the inspiratory rib cage muscles is linear whereas that of the diaphragm is concave towards EMG-axis at high EMG activity, indicating that when contracting alone the ability of the diaphragm to generate volume change is decreased especially at high drive whereas that of the rib cage muscles is not. In keeping with this, the inspiratory rib cage muscles shorten much less than does the diaphragm over a given volume change (68,251) and a given increase in EMG activity (251). Finally and most importantly, rib cage muscle recruitment improves the inspiratory action of the diaphragm so that a greater fraction of the Pdi developed is converted to a useful fall in pleural pressure (163). This suggests that with increasing drive, the diaphragm as a major inspiratory muscle needs more and more assitance from the rib cage muscles. When both the diaphragm and the rib cage muscles are activated together, the major work done by these two inspiratory muscles is to inflate the rib cage and lung. As shown by Hussain and coworkers (124), rib cage strapping markedly reduced exercise endurance whereas abdominal strapping did not.

It has been noticed that expiratory muscle (especially abdominal muscle) contraction is frequently seen during expiration with chemical and exercise stimulated breathing (1,112,115,156,160,245,252) or inspiratory resistive loading (1,6,126,137,156,171,173,180,214). This expiratory muscle action not only facilitates expiration but also assists and even directly contributes to inspiration by two mechanisms. Firstly, abdominal

muscle contraction during expiration decreases end expiratory lung volume. The diaphragm thus contracts at longer initial length and works as a better inspiratory pressure generator. Secondly, the relaxation of abdominal muscles at the beginning of subsequent inspiration may release the elastic energy stored in the chest wall which initiates inspiration to FRC without the need of inspiratory muscle activation. Furthermore, during inspiration, abdominal pressure may not change so that the abdominal wall acts as a compartment with infinite compliance (115). This expiratory muscle contribution was reported to be as high as ~65% of tidal volume in head-up dogs (87,89). In humans, Grassino et al (112) reported that when the ventilation is stimulated by CO_2 there is an "inspiratory dip" of abdominal pressure at the beginning of inspiration which is associated with inspiratory rib cage and abdomen movement and inspiratory flow; in the first 100ms of occluded inspiration, the change in Pdi is substantially smaller than that of Pm. These authors therefore concluded that inspiratory pressure can be partially generated by abdominal muscle relaxation. Similar findings were also reported by Lopata et al (156) and Abbrecht et al (1) who demonstrated a greater contribution of abdominal muscle relaxation to inspiratory displacement of the chest wall during inspiratory resistive loaded breathing. With the benefit of abdominal muscle participation in the inspiratory process, the required inspiratory muscle activity is presumably reduced for a given level of ventilation.

As has been discussed, with an increased ventilatory task or

under load, the respiratory muscles respond as a group to recruit inspiratory rib cage muscles proportionately more than the diaphragm and progressively recruit expiratory muscles. With respiratory muscle fatigue, the load the inspiratory muscles contract against is increased relative to strength for a given level of ventilation. Thus a compensatory change in the recruitment pattern of respiratory muscles might be expected. Bellemare and Grassino (23) observed that COPD patients could maintain or even increase Pes swing and tidal volume and decrease breathing frequency with an imposed breathing pattern but at the cost of development of diaphragmatic fatigue. In this study, a decreased Pdi swing with a well-maintained Pes change during breathing must indicate recruitment of inspiratory rib cage muscles as a compensatory mechanism (163). This result indicates that compensatory recruitment of inspiratory rib cage muscles and presumably expiratory muscles may be important determinants of ventilation and breathing pattern after fatigue. However, although it was previously suggested that the coordination and synergism of respiratory muscles contracting against load might protect against fatigue and delay task failure (157,225), there has been no systematic studies to describe how respiratory muscle fatigue affects respiratory muscle recruitment pattern and what impact the changes in recruitment has on ventilatory response. These are important issues of respiratory muscle fatigue and control of breathing and need detailed and systematic investigations.

1.4 Summary

After extensive studies of recent years, we have learnt a great deal of respiratory muscle function and fatigue. We now know that there are similarities between respiratory muscles and other skeletal muscles in many respects; we know that respiratory muscles can be fatigued at least in laboratories when doing excessive work; we know that the respiratory muscles of patients may have increased work, decreased strength and energy supplies rendering the development of fatigue likely; we also know that compensatory mechanisms may exist to prevent or delay the development of respiratory muscle fatigue in patients. But we still do not know how frequently if at all respiratory muscle fatigue exists in patients because we have no a reliable test to detect respiratory muscle fatigue. We are also not sure that if fatigue occurs, how it would influence the recruitment pattern of respiratory muscles and how changes in respiratory muscle contractility would influence ventilation and its control.

In trying to answer these questions, the work presented in this thesis has been designed as two parts. The first part composed of chapter 2 through chapter 4 evaluates the value of transcutaneous supramaximal bilateral phrenic nerve stimulation as an objective test to assess respiratory muscle fatigue. The second part containing chapter 5 and chapter 6 evaluates the effect of diaphragmatic fatigue and global inspiratory muscle fatigue on respiratory muscle recruitment and ventilatory control. All the studies are performed on normal human subjects.

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CHAPTER 2

EFFECT OF FATIGUE ON DIAPHRAGMATIC FUNCTION AT DIFFERENT LUNG VOLUMES

It has been proposed that measurement of diaphragmatic twitches in response to bilateral supramaximal transcutaneous phrenic nerve stimulation may have potential as a clinical test to detect diaphragmatic fatigue. Such a test is badly needed because: [1] fatigue is thought to be a common cause of hypercapnic respiratory failure; [2] no acceptable diagnostic test is available to detect fatigue; [3] thus there is no reliable statistics on the prevalence of fatigue as a cause of ventilatory failure; [4] the importance of inspiratory muscle fatigue as a medical problem remains underdetermined. However, before applying this technique to patients, more information needs to be obtained. For example, it is known that the amplitude of diaphragmatic twitches is dependent on lung volume. This is largely explained by the muscle length-tension relationship. However, how fatigue affects this relationship remains unexplored. This information is necessary before phrenic nerve stimulation becomes a diagnostic test and is provided by the study presented in this chapter.

2.1 Abstract

The transdiaphragmatic pressure twitches (Pdi,T) in response to single supramaximal shocks delivered bilaterally to the phrenic nerves were recorded as a function of lung volume when the diaphragm was fresh and when fatigued. All relationships were linear and negatively sloped (all $r > 0.85$). From these relationships Pdi,T was found to decrease with fatigue more rapidly and to recover more quickly at high than at low lung volumes. Complete recovery of Pdi,T at all lung volumes was > 1 hour. Contraction and relaxation rate constants of Pdi,T did not change significantly with fatigue. I conclude that fatigue affects diaphragm contractility more at high than at low lung volumes and that changes in diaphragm contractility are best reflected in the measurement of Pdi,T as a function of lung volume.

Key words: diaphragm; muscle fatigue; phrenic nerve stimulation; contractile properties; twitch

2.2 Introduction

It was recently proposed (19) that supramaximal transcutaneous bilateral phrenic nerve stimulation may possibly become a useful diagnostic test of diaphragmatic fatigue. Such a test is badly needed to determine if respiratory muscle fatigue is clinically relevant. The present work is part of a series of investigations with the ultimate objective of developing this method to detect diaphragmatic fatigue in patients. One of the requirments for achieving this objective is to determine the influence of lung volume as a variable on indexes of diaphragmatic contractility obtained from bilateral transcutaneous supramaximal phrenic nerve twitches before and after fatigue and to determine the recovery rate of these indexes as a function of lung volume. By doing so I hoped to make this study more than the development of a technique, I wished to obtain new physiological information on the effect of lung volume on diaphragmatic fatigue and its recovery in vivo in normal humans. In particular, it has been reported in vitro that diaphragmatic strips, when fatigued, have a disproportionately greater loss of force at short than at long lengths (10). I wished to determine if this is also the case in humans in vivo. Phrenic nerve twitches have shown that human diaphragmatic fatigue is characterized by a decrease in diaphragmatic contractility (3,5). In this paper, I define contractility as the contractile response of the diaphragm to a bilateral supramaximal phrenic nerve shock and assessed it as the peak transdiaphragmatic pressure twitch amplitude (Pdi,T) since Pdi,T measurement has been shown to be

reproducible for a given condition in normal subjects (4). I also assessed contractility as the time it takes to develop maximal force, the rate of rise of force, and the relaxation characteristics. Twitch Pdi measured in response to bilateral supramaximal phrenic nerve stimulation is inversely related to lung volume (12,18,21). Thus, if one wishes to use phrenic nerve stimulation to detect fatigue it is necessary to take lung volume into account. Moreover, it has been shown that diaphragmatic endurance of humans in vivo (20,23) and of animals in vitro (8) varies substantially at different lung volumes or at different muscle fibre length. It was therefore the purpose of the present study to determine in normal subjects how fatigue and recovery influence contractility at different lung volumes using supramaximal bilateral phrenic nerve twitch stimulation.

2.3 Methods

Subjects and measurements:

Five normal male laboratory volunteers (mean age 32.4±5.1SD yr) were studied seated. All gave their informed consent. Mouth pressure (Pm), esophageal pressure (Pes) and gastric pressure (Pga) were recorded conventionally (1,17). Pdi was defined as Pga-Pes. Lung volume was measured by a bag-in-box system consisting of a meteorological balloon placed in a volume displacement body plethysmograph and connected to the inspiratory side of a one way valve. The expiratory side of the valve was connected to the plethysmograph. Anteroposterior dimensions of upper rib cage (APrc) and abdomen (APab) were measured with two pairs of magnetometers positioned in the midline, one pair 2-3 cm below the angle of Louis and the other pair 2 cm above umbilicus, respectively, according to the method of Konno and Mead (14).

Phrenic nerve stimulation:

The methods employed in this study to stimulate supramaximally the phrenic nerves bilaterally in the neck and to record the evoked muscle mass action potentials (M-waves) of the diaphragm have all been described in detail elsewhere (4).

Protocol:

At the beginning of each test, the configuration of the relaxed chest wall was established first by recording APrc and APab on a Konno-Mead diagram during successive stepwise deflations starting at TLC. This procedure was repeated until the chest wall configuration thus obtained was reproducible. This relaxed

configuration was marked on the screen of an oscilloscope and used as a reference throughout the remainder of the test to ensure that subsequent measurements were obtained under as relaxed conditions as possible. Five or six different lung volumes nearly equally spaced between TLC and FRC were then identified on the relaxation diagram and marked on the oscilloscope. Starting at TLC and with the subjects relaxed, at least 5 diaphragmatic twitches evoked by supramaximal bilateral phrenic nerve single shocks were then recorded with the airways occluded close to each of the designated lung volumes. The same procedure was also repeated soon after the fatigue run (see below) and after 15, 30, and 60 minutes of rest.

To induce diaphragmatic fatigue, the subjects were asked to breathe through an inspiratory resistance adjusted so as to enable the subjects to develop, with each breath, 60% of maximal Pdi, which was displayed to the subject on a storage oscilloscope, until the target could no longer be maintained for several consecutive breaths, and the subject came off the mouthpiece.

Data recording and analysis:

The pressure, magnetometers, and lung volume signals were all recorded on an eight-channel chart recorder. The pressure, magnetometer signals, and the M-waves from left and right hemidiaphragms were also recorded on tape. These were subsequently fed through a 12 bit analog to digital converter to a computer for further analysis. The sampling rate of the computer was set at 1500 Hz for EMG signals and 500 Hz for pressure and magnetometer signals. Five of the Pes, Pga, and Pdi twitches (Pes,T, Pga,T,

Pdi,T) were averaged at each occluded lung volume. The peak twitch amplitude was measured from the averaged signals after subtracting the immediately preceding baseline. The M-waves corresponding to these twitches from each hemidiaphragm were isolated and averaged in the same way and their peak height measured in arbitrary units relative to the isoelectric line. Standard linear regression techniques using the least square method were employed to evaluate the changes in M-wave and Pdi,T with changes in lung volume. The slopes and intercepts before and after fatigue or recovery were compared using a paired t test. The statistical level of significance was taken as $p<0.05$. Group values were expressed as Mean$\pm$SE.

2.4 Results

As shown in figure 2.1, the relaxation volume-pressure relationships of the total respiratory system (A), the chest wall (B), and its abdominal and rib cage compartments (C and D) were all comparable before and after the fatigue runs.

Figure 2.2 shows that Pdi,T decreased at all lung volumes with fatigue. By contrast, the M-waves from both hemidiaphragms did not change with fatigue at any given lung volume. Pdi,T decreased linearly with increasing lung volume in all subjects and conditions studied (all $r > 0.85$). Pdi,T could thus be calculated from the regression at different lung volumes and expressed as percent of the value calculated at that volume when the diaphragm was fresh. As shown in figure 2.3, the fractional decrease in Pdi,T with fatigue was greater and recovered faster at high than at low lung volumes; however, at all lung volumes, Pdi,T was still depressed after 60 min. recovery.

Pga,T and Pes,T decreased in nearly the same proportion with fatigue. Accordingly, the Pes,T/Pdi,T ratio, which decreased with increasing lung volume (from 0.47 ± 0.03 at FRC to 0.31 ± 0.05 at TLC), did not change with fatigue at any given lung volume.

The time-related properties of Pdi,T (twitch contraction and relaxation times and maximal rate of contraction and relaxation) were also measured; however, no change was found with fatigue at any given lung volume and the results were not shown here.

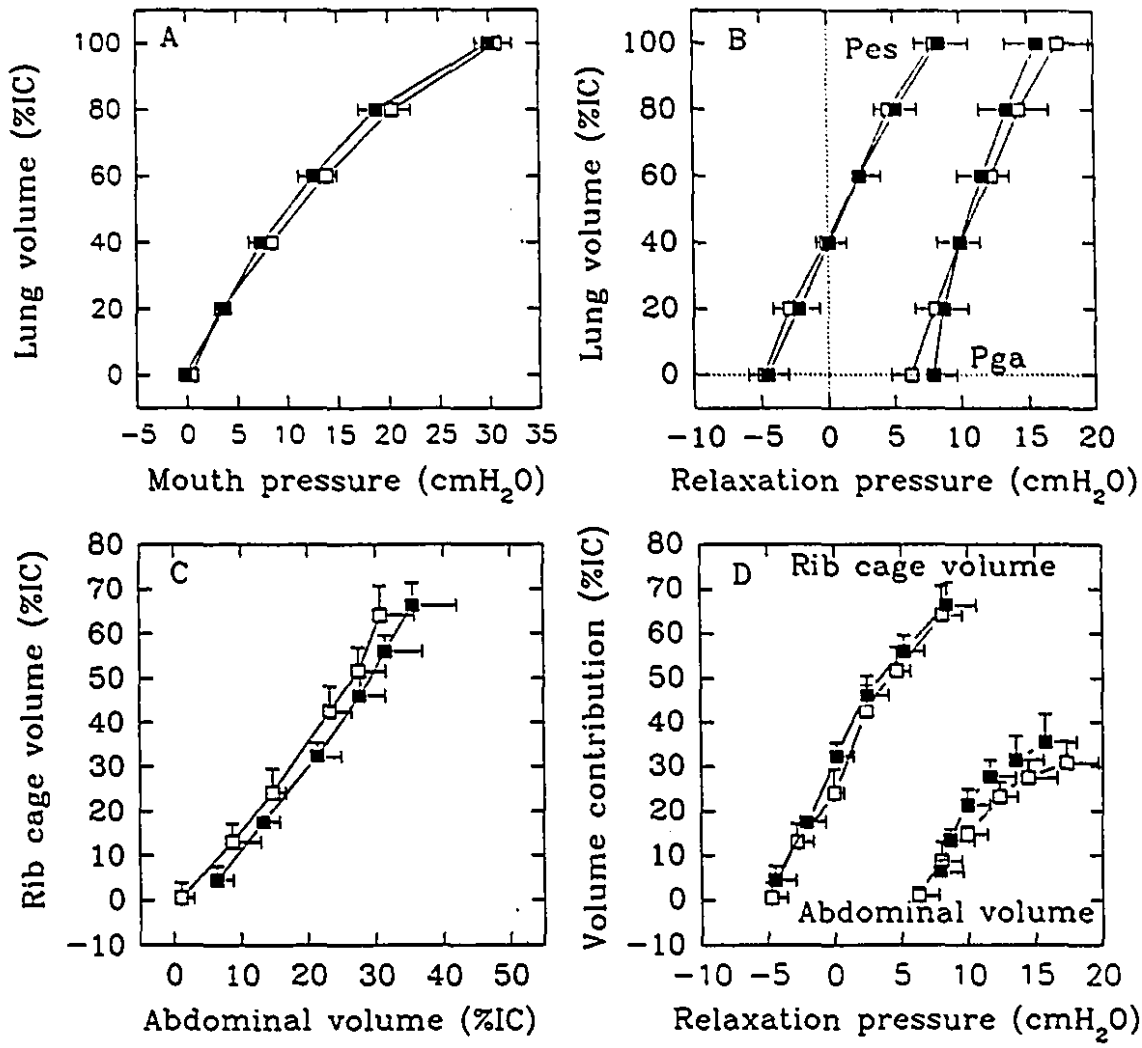

Figure 2.1: Relaxation characteristics. A: volume-pressure relationships of total respiratory system. B: volume-pressure relationships of chest wall. C: chest wall configuration on Konno-Mead diagram, D: volume-pressure relationships of the rib cage and abdomen. Open squares, before fatigue; Closed squares, after fatigue. Lung volume, rib cage volume, and abdomen volume are expressed as percent of inspiratory capacity (%IC).

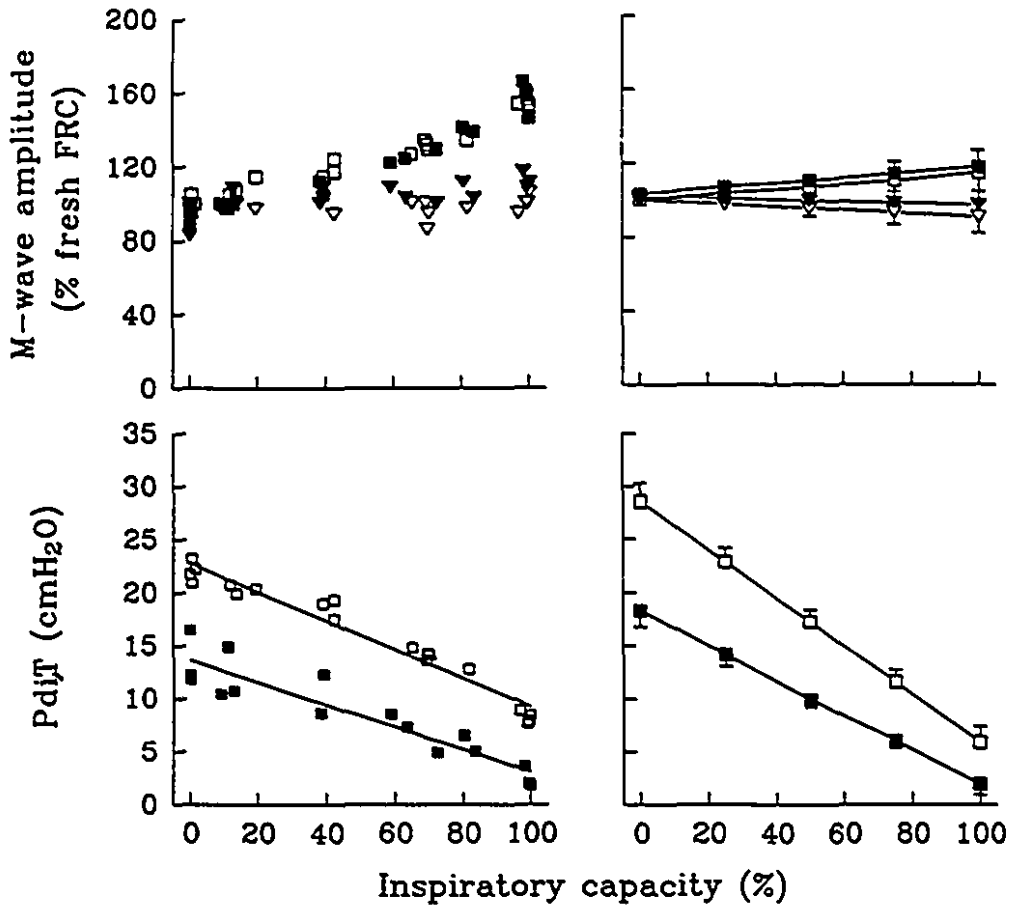

Figure 2.2: Relationships of M-wave amplitude and transdiaphragmatic pressure twitch (Pdi,T) to lung volume. Left panels: results from 1 representative subject; right panels: group results calculated from linear regressions for each subject. Lung volume is expressed as percent inspiratory capacity. M-wave amplitude is normalized as percent of that measured at functional residual capacity (FRC) in fresh condition. Open symbols, before fatigue; closed symbols, after fatigue. For top panels, triangles denote the M-wave amplitude recorded over the left, and squares those recorded over the right hemithorax.

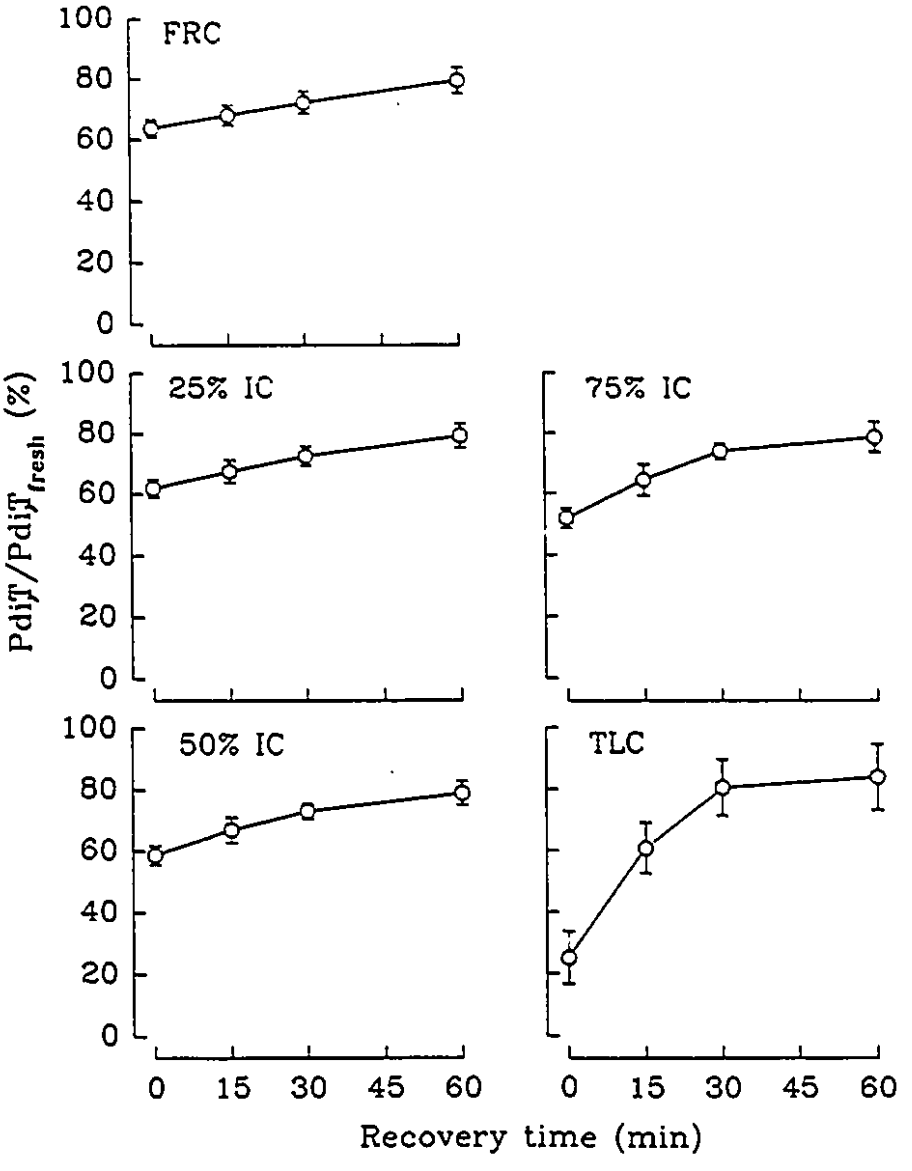

Figure 2.3: Pdi,T amplitude with fatigue and during recovery at 5 different lung volumes (%IC). Pdi,T is expressed as fraction of PdiT before fatigue at each lung volume. The time zero corresponds to the time of the first post fatigue determination.

2.5 Discussion

I found that Pdi,T decreases linearly with increasing lung volume and that this relationship remains linear with fatigue, thereby providing a simple means of evaluating changes in diaphragmatic contractility in vivo as a function of lung volume. Other indexes of diaphragm contractility such as the velocity characteristics of the twitch showed no systematic changes with fatigue, presumably because of their rapid recovery (7,15), and therefore were less useful in evaluating changes in diaphragm contractility than Pdi,T.

I recognize that the Pdi,T versus lung volume relationship could also be affected by changes in configuration of the chest wall independent of changes in diaphragm contractility (11,13,16). In this study, the chest wall configuration was continuously monitored with magnetometers during relaxation. I showed that fatigue did not influence the elastic properties of the respiratory system and thus did not affect the relaxation configuration. As chest wall configuration determines diaphragmatic fibre length at any given lung volume (11), and the configuration of chest wall at a given lung volume was maintained unchanged with fatigue, fibre length of both costal and crural parts of the diaphragm before the twitch whether fresh or fatigued should be uniquely related to lung volume. This factor therefore should not have significantly influenced my results. Furthermore, the study of Hubmayr et al (12) suggested that the effect of lung volume changes on Pdi,T is paramount as compared to that produced by changes in chest wall

shape.

From the relationships between Pdi,T and lung volume I found a greater fractional decrease of Pdi,T at high than at low lung volumes with fatigue, which recovered quickly. I am not aware of any data to which these results can be directly compared. Studies on human limb muscles in vivo (9) as well as on amphibian limb muscles (2) and hamster diaphragm (8) in vitro reported a greater susceptibility to fatigue when this was induced at a longer length as compared with a shorter length. In as much as the diaphragm shortened with increasing lung volume in my subjects (6,21), my results are opposite to the other results. The discrepancy most probably results from the different fatigue protocol employed. In these other studies, the muscles were fatigued at different lengths generating different tension and presumably causing different metabolic changes in the muscle. In the present study the diaphragm was fatigued at a given lung volume and only the pre- and post-fatigue measurements were performed at different lung volumes and fibre length. In line with this expectation, Farkas et al (8) demonstrated in hamsters, a more rapid decay of diaphragm force in vitro at shorter as compared with at longer length when attempting to match the force at both lengths by adjusting the stimulation frequency.

More recently, a disproportionate decrease in tetanic tension at short length as compared to rest length has been reported for the rat diaphragmatic strips isolated in vitro when fatigued at the rest length causing a shift in length-tension curve to the

right along the length axis (10). The mechanism(s) underlying these changes in length-tension properties of the diaphragm with fatigue are incompletely understood, but if such changes also occur in vivo, they would explain my results. One possible mechanism could be that the well known failure of muscle activation at short length was exaggerated by fatigue because of impaired inward spread of the action potentials in the tubular system (22). The rapid recovery from fatigue at high lung volumes is also suggestive of an impaired depolarization.

Whether these results are also relevant to the clinical manifestation of diaphragmatic fatigue remains to be assessed. However, earlier studies have shown that increasing lung volume reduces the endurance of the inspiratory muscles (20,23). To the extent that the shorter endurance can be linked to a more rapid decay of diaphragm contractility at high lung volumes, then my results are relevant. Furthermore, to the extent that airways obstruction may lead simultaneously to both fatigue and acute hyperinflation, the increase in lung volume might lead to a viscious circle both by decreasing strength and by aggravating the effects of fatigue. Finally, by extrapolating figure 2.2 the lung volume at which the diaphragm would be unable to develop any pressure is diminished by fatigue.

In summary, the highly significant inverse linear relationship between Pdi,T and lung volume was employed as a means to evaluate changes in diaphragmatic contractility caused by fatigue in normal human subjects. The most clear-cut change related to fatigue was

the decreased Pdi,T amplitude at all lung volumes with unchanged diaphragmatic M-waves. However, this did not affect the inspiratory function of the diaphragm. The changes of time-related properties of Pdi,T were less significant. The results also disclosed that fatigue preferentially decreased diaphragm pressure development at high lung volumes, compared with smaller ones. Recovery from this volume effect was rapid. The underlying mechanism may be related to impaired propagation of sarcolemmal depolarization along the tubular system. Despite the rapid recovery from this component of fatigue, complete recovery of diaphragm function was longer than 1 hour at all lung volumes.

2.6 References

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CHAPTER 3

EVALUATION OF HUMAN DIAPHRAGM CONTRACTILITY USING MOUTH PRESSURE TWITCHES

In chapter 2, it was demonstrated that the measurement of Pdi twitch amplitude in response to phrenic nerve stimulation can assess the changes in diaphragmatic contractility caused by diaphragmatic fatigue when the influence of lung volume is taken into account. However, to measure Pdi requires placement of esophageal and gastric balloon-tipped catheters. This procedure is invasive and not easily accepted by all patients. The research in this chapter was designed to test whether or not the changes in diaphragmatic contractility as a result of the variation in lung volume or fatigue can be satisfactorily determined by measuring mouth pressure twitches during phrenic nerve stimulation against a closed airway.

3.1 Abstract

Mouth (Pm,T), esophageal (Pes,T), and transdiaphragmatic pressure twitches (Pdi,T) in response to single supramaximal bilateral phrenic nerve shocks were recorded during relaxation between total lung capacity (TLC) and functional residual capacity (FRC) in 5 normal volunteers. The Pm,T versus Pes,T or Pm,T versus Pdi,T relationships, which were linearly correlated (all $r > 0.76$), were not affected by diaphragm fatigue and were reproducible on repeated determinations over a period exceeding one year. The Pm,T versus lung volume relationship was also linear (all $r > 0.72$) and reproducible, and its changes following diaphragm fatigue reliably reflected the changes in diaphragm contractility. I conclude that Pm,T is a reliable measure of diaphragm pressure generating capacity in normal individuals and has the potential of providing a similar information in clinical patients.

Key words: diaphragm; twitch; mouth pressure; lung volume; muscle fatigue; bilateral phrenic nerve stimulation

3.2 Introduction

It was recently proposed (19) that bilateral transcutaneous supramaximal phrenic nerve twitch stimulation would have the potential of eventually becoming a diagnostic test for evaluating diaphragmatic muscle fatigue. To achieve this goal I have conducted a series of investigations ultimately to develop a non-invasive test of diaphragmatic contractility which I hope will be useful in detecting diaphragmatic fatigue and determining its prevalence. Using this technique, recent studies on normal human diaphragm in vivo have shown that transdiaphragmatic pressure twitch (Pdi,T) amplitude decreases linearly with increasing lung volume (21). The relationship remains linear following diaphragmatic fatigue, although shifts to lower Pdi,T values (23).

The major advantage of bilateral phrenic nerve stimulation is its independence of subject's motivation and cooperation. When single shocks are applied, this technique is essentially painless and can be achieved non-invasively (5,6). However, the invasive nature of transdiaphragmatic pressure measurements with the balloon tipped catheters (2,15) may limit the clinical application of this method. Therefore, in order to capitalize on the Pdi,T-lung volume relationship following fatigue as a diagnostic test, I determined in the present research if diaphragmatic contractility could be assessed simply by measuring the mouth pressure swing against an occluded airway in response to phrenic nerve twitch stimulation (19).

With the glottis open and the airways closed at the mouth,

mouth pressure is a good estimate of the overall pleural surface pressure change if the time constant for equilibration of alveolar and mouth pressure is sufficiently short. I show that this is the case in normal lungs and preliminary study (20) indicates that it is also the case in severe chronic obstructive pulmonary disease (COPD) if the patients perform a gentle Meuller manoeuvre against the occluded airways. Furthermore, when the diaphragm is the only muscle contracting, such as with phrenic nerve stimulation, I show that mouth pressure swings closely reflect the pattern of transdiaphragmatic pressure change. The purpose of this study, therefore, was to determine the relationships between mouth pressure twitch (Pm,T) on the one hand and esophageal pressure twitch (Pes,T), Pdi,T, and lung volume on the other in fresh and fatigued diaphragms of normal subjects.

3.3 Methods

Subjects and measurements

Five normal male laboratory volunteers (mean age 33±6 SD yr) were studied in the seated position. Informed consent was obtained from all subjects. Esophageal pressure (Pes) and gastric pressure (Pga) were measured separately with two conventional balloon-tipped catheter systems and differential pressure transducers (Honeywell 8604) as described previously (2,15). Pdi was used to reflect changes in muscle tension and was obtained as Pes-Pga, after subtracting the value obtained during relaxation at FRC (2). A third transducer was employed to measure mouth pressure, sensed at the mouthpiece. Lung volume was obtained by integrating the flow signal from a pneumotachograph (Fleish #2; Instrumentation Associates, Inc., New York, NY).

Phrenic nerve stimulation

The method of bilateral transcutaneous supramaximal phrenic nerve stimulation and of recording the elicited diaphragm muscle mass action potentials (M-waves) with surface electrodes has been described in detail previously (5,6).

Protocol

Diaphragmatic twitches were recorded during passive deflations from total lung capacity (TLC) to functional residual capacity (FRC). Subjects were assumed to be relaxed when doing such a manoeuvre. They were instructed to inspire to TLC and to exhale passively through a high resistance placed on the expiratory side of a two way valve. The inspiratory side of the valve was

obstructed during this manoeuvre. During this slow exhalation, supramaximal shocks were delivered bilaterally to the phrenic nerves at a rate of about 0.5-1.0 Hz. In one subject, the stimuli were delivered during brief (5s) periods of airway occlusion at several different lung volumes between TLC and FRC. The results were the same using either method, and therefore will not be reported separately.

Validation experiments:

Effect of paired stimuli. In 2 subjects, the measurements were repeated using paired stimuli instead of single shocks. The interval between the 2 stimuli of each pair was varied in different runs between 25 and 100 ms. They served to prolong the contraction of the diaphragm and thus to verify whether the single twitch was sufficiently long to allow equilibration of mouth pressure with alveolar pressure.

Effect of diaphragm contraction on pleural pressure gradients. In 2 subjects, Pes was recorded at 3 different heights using one additional balloon-catheter system of identical size. This balloon was positioned in different runs at either 5 or 15 cm above the gastro-esophageal junction. The other esophageal balloon remained in the standard position at 10 cm above junction. Diaphragmatic twitches were recorded as described above.

Reproducibility of the measurements. Reproducibility of the Pm,T versus Pes,T and Pdi,T relationships was evaluated in 4 subjects who were studied each on 3 separate occasions over a period exceeding one year. Reproducibility of the Pm,T versus lung

volume relationship was separately evaluated in 3 subjects who were studied each on 3 separate days over a period of 2 weeks. On each testing day, 2 testing runs were made.

Effect of changes in diaphragm contractility. In the 5 subjects studied, diaphragmatic twitches were also recorded after the contractility of their diaphragm was reduced by fatigue. Fatigue was induced by breathing against an inspiratory resistance (about 80-100 $cmH_20/L/sec$) adjusted in such a way as to enable the subjects to develop with each breath 60% of their maximal Pdi with a duty cycle of 0.6. This test was conducted until the limit of endurance after which the diaphragmatic twitches were recorded as described above.

Data recording and analysis:

The pressure and volume signals were recorded on a strip chart recorder. The pressure signals and the two M-waves from left and right hemidiaphragms were also recorded on tape. Lung volumes were measured from the paper recordings using TLC as the reference volume and expressed as % of inspiratory capacity (%IC). Analysis of the pressure signals and M-waves was done on tape playback by feeding the signals through a 12 bit analog to digital converter to a computer. The sampling rate was set at 2000 Hz for the M-waves and 500 Hz for the pressure signals. All the twitches for which the M-waves amplitude from both sides remained maximal were retained for analysis. For all Pdi, Pm and Pes twitches, peak amplitude was measured relative to the immediately preceding baseline. For all the relationships presented in this paper, standard regression

techniques were employed. A paired t test was used to compare the slopes and intercepts before and after fatigue and GLM-Anova analysis (General linear models analysis of variance or covariance) was employed to test the reproducibility of Pm,T measurement. A $p < 0.05$ was considered statistically significant. Dispersion is represented by the standard error of the mean (SEM).

3.4 Results

One representative experimental record of Pm, Pes, and Pga twitches at different lung volumes is shown in figure 3.1. The diaphragmatic twitches are clearly detected as a sharp negativity in the Pm and Pes records and a sharp positivity in the Pga record.

Transient overshoots were noted in Pm and Pes tracings following a twitch, which coincided with a delayed relaxation in the Pga tracing. These overshoots were observed in all subjects, albeit to a variable extent. Presumably they reflect abdominal muscle contractions elicited reflexly or otherwise. Because of their transient character and their occurrence late during the relaxation phase of the twitch, however, these overshoots did not interfere with the measurement of peak Pm,T, Pes,T, and Pga,T.

The relationships between Pm,T and Pes,T and between Pm,T and Pdi,T for all subjects and conditions examined in this study are shown in figure 3.2. In all cases, the Pm,T versus Pes,T relationships were linear, and in the four subjects the Pm,T versus Pdi,T relationships were also linear (all $r > 0.76$). For the remaining subject, the Pm,T versus Pdi,T relationships showed a curvilinearity in the Pdi,T range of -25 to -40 cmH_2O, accounting for the curvilinearity seen in figure 3.2. Even for this subject, however, a linear model provided a reasonably good fit ($r = 0.91$) compared with a curvilinear model (power function; $r = 0.95$) and also favorably compared with that of the other subjects (range of r 0.85 to 0.93). for this reason, in the following comparison, a linear model was employed in all subjects and conditions:

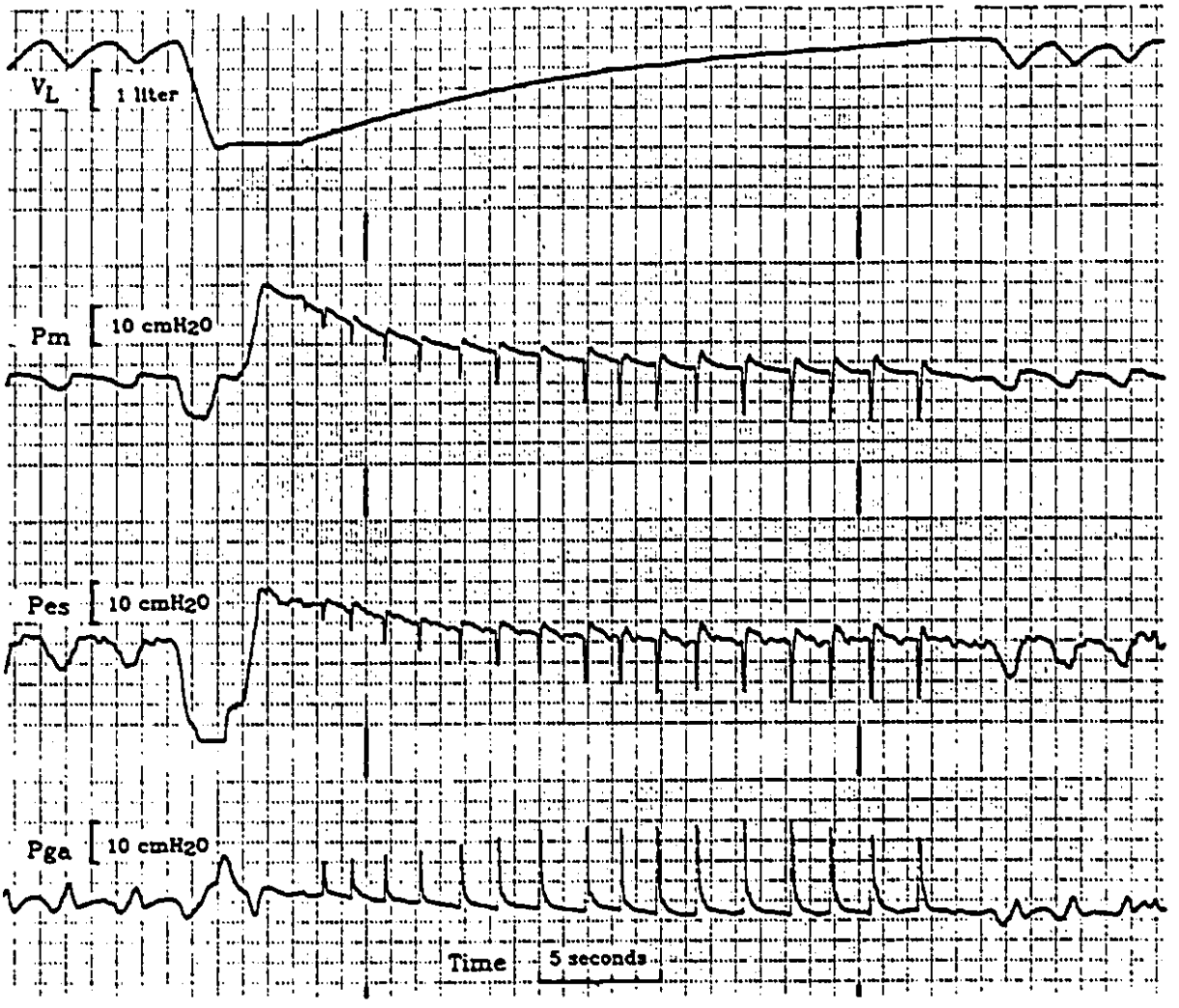

Figure 3.1: Representative experimental recording of the changes in lung volume (V_L), mouth pressure (Pm), esophageal pressure (Pes), and gastric pressure (Pga) for one subject before fatigue.

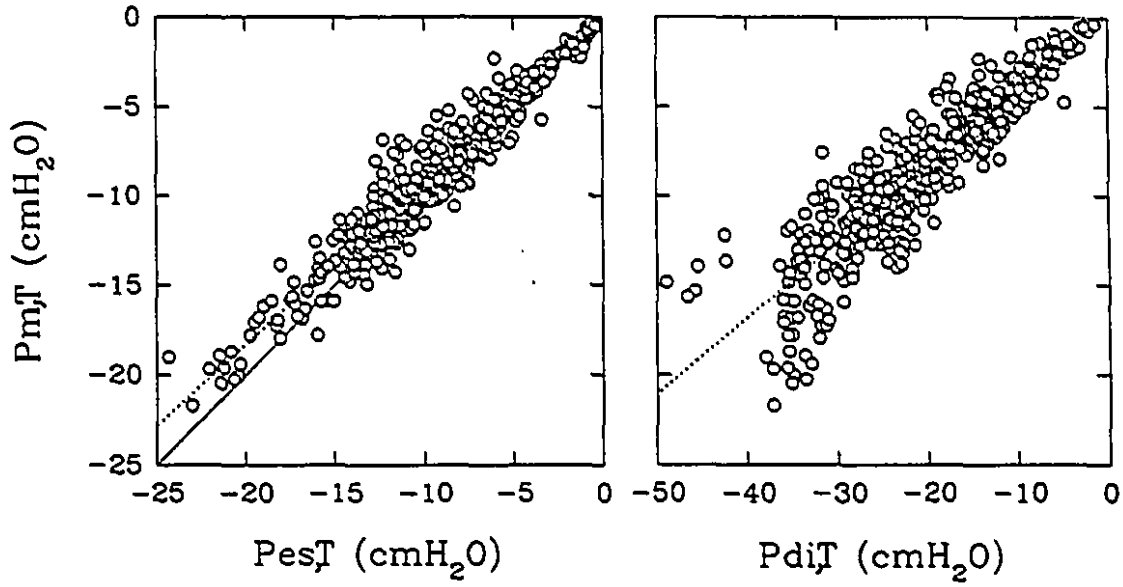

Figure 3.2: Mouth (Pm,T) versus esophageal pressure twitch (Pes,T) diagram (left) and Pm,T versus transdiaphragmatic pressure twitch (Pdi,T) diagram (right) of the data points from all experimental conditions of 5 subjects. Oblique line is line of identity (left). Dotted line of each diagram is the linear regression through each set of data.

$$Pm,T = a + b * Pes,T \quad [1]$$

$$Pm,T = a' + b' * Pdi,T \quad [1']$$

For all subjects studied, the value of the constants in equation 1 and equation 1' did not differ significantly on repeated trials when performed the same day (for example, before and after a diaphragm fatigue run) or on different days, even though these were separated by up to 16 months (table 3.1). Furthermore, the values of a and a' for the group were not significantly different from zero.

For the group data, Pm,T was significantly smaller than Pes,T ($p<0.05$), and the standard deviation of the differences between Pm,T and Pes,T was 1.24 cmH_2O. As a result, the value of coefficient b in equation 1 of 0.88 ± 0.09 for the group was significantly smaller than 1. As shown for two subjects in figure 3.3A, increasing the duration of the diaphragmatic contractions by using paired stimuli of varied intervals instead of single shocks, had no effect on the Pm,T versus Pes,T relationship and hence on the value of b. However, systematic regional differences were found in PesT values depending on the site of recording, more negative values being obtained at distal as compared to proximal recording sites (figure 3.3B).

For the 5 subjects, Pm,T and Pdi,T both decreased linearly with increasing lung volume (all $r > 0.72$). This is shown for one subject in figure 3.4. In all subjects, these relationships were shifted upward after fatigue, indicating an impaired diaphragmatic contractility. The analysis of covariance showed that, when

corrected for lung volume changes, Pm,T decreased with fatigue in nearly the same proportion as Pdi,T and both changes were highly significant (table 3.2).

Table 3.1

Reproducibility of Pm,T vs Pes,T and Pm,T vs Pdi,T relationships.

	Pm,T = a + b * Pes,T (cmH_2O)		Pm,T = a'+ b'* Pdi,T (cmH_2O)	
	a	b	a'	b'
day 1 (n=4)	-0.84±0.34	0.98±0.15	3.99±1.98	0.67±0.09
day 2 (n=4)	-1.05±0.60	0.87±0.08	2.83±1.94	0.52±0.06
day 3 (n=4)	-1.40±1.16	0.97±0.27	3.90±3.14	0.56±0.12
Fresh (n=5)	-0.04±0.34	0.86±0.05	1.27±0.45	0.45±0.05
Fatigue (n=5)	-0.43±0.25	0.83±0.06	0.45±0.49	0.42±0.08

The relationship between Pm,T and lung volume was highly reproducible in the three subjects in whom this was tested. Analysis of covariance showed that when corrected for lung volume changes, Pm,T, which differed significantly among the subjects, did not differ significantly on repeated trials whether performed the same day or on different days in the same subject (table 3.3).

The size of the diaphragmatic M-waves in response to maximal phrenic nerve stimuli sometimes varied considerably when measured on different days in the same subject. Their amplitude also varied

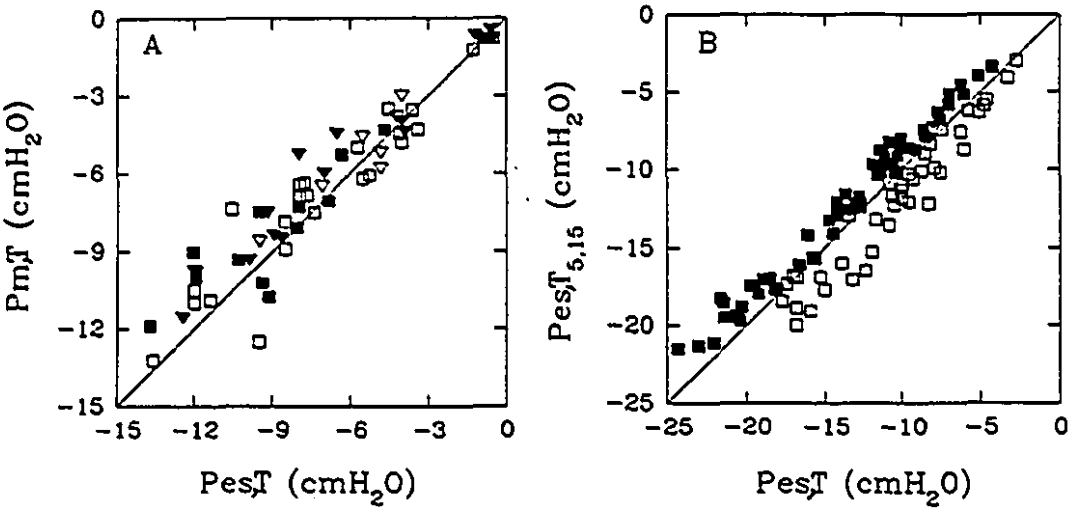

Figure 3.3: (A) Pm,T versus Pes,T diagram of 2 subjects obtained with paired stimuli of different intervals: 100ms (open triangles); 66ms (closed triangles);50ms (open squares); and 25ms (closed squares). (B) Pes,T measured from 2 subjects at 5 cm (open squares) and 15 cm (closed squares) above gastroesophageal junction (y-axis) plotted as a function of Pes,T recorded at standard position (x-axis). Oblique lines are identity lines.

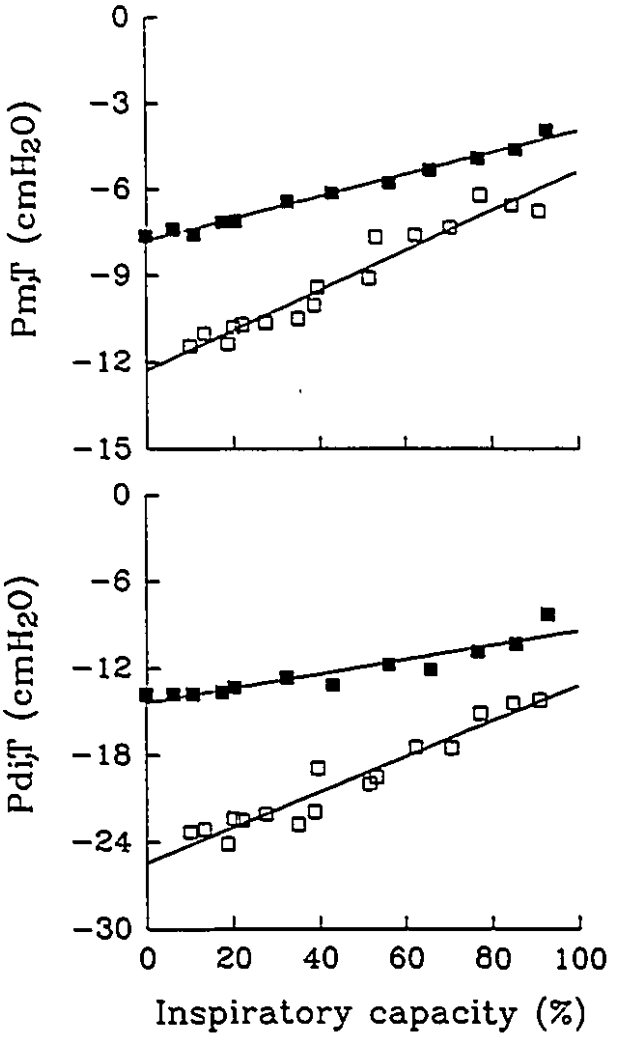

Figure 3.4: Relationships between Pm,T and lung volume and between simultaneously recorded Pdi,T and lung volume expressed as percent inspiratory capacity (%IC) in one representative subject before (open squares) and after diaphragmatic fatigue (closed squares). Linear regression lines are drawn through each set of data.

Table 3.2

Adjusted mean Pm,T and Pdi,T amplitude for the fitted linear models affected by subjects and fatigue

Factors	Pm,T (cmH_2O)	P value	Pdi,T (cmH_2O)	P value
Subjects				
1	-3.53±1.21		-11.73±1.14	
2	-7.99±0.96		-13.49±1.44	
3	-5.13±0.89	0.15	-13.91±1.06	0.26
4	-5.18±0.84		-11.74±1.04	
5	-7.23±0.79		-15.22±0.94	
Conditions				
Fresh	-7.43±0.16	< 0.0001	-16.89±0.28	< 0.0001
Fatigue	-4.19±0.19		-9.55±0.33	

TABLE 3.3

Reproducibility of the adjusted mean Pm,T amplitude for the fitted linear models affected by test runs, test days, and subjects.

Factors tested	Corrected Pm,T Amplitude (cmH_2O)	P value
Test runs		
1	-9.75+0.17	0.443
2	-9.93+0.18	
Test days		
1	-9.76+0.21	
2	-10.07+0.21	0.397
3	-9.69+0.21	
Subjects		
1	-7.75+0.14	
2	-11.13+0.15	< 0.0001
3	-10.56+0.12	

between the left and right sides in any given subject as well as with changes in lung volume, however, no consistent pattern was observed. By contrast, the size of the diaphragmatic M-waves was highly reproducible at any given lung volume when measured on repeated trials performed the same day. This is shown for one subject in figure 3.5. A similar within day reproducibility was obtained in all subjects, irrespective of whether the comparison was made on the fresh diaphragm or between the fresh and fatigued diaphragm.

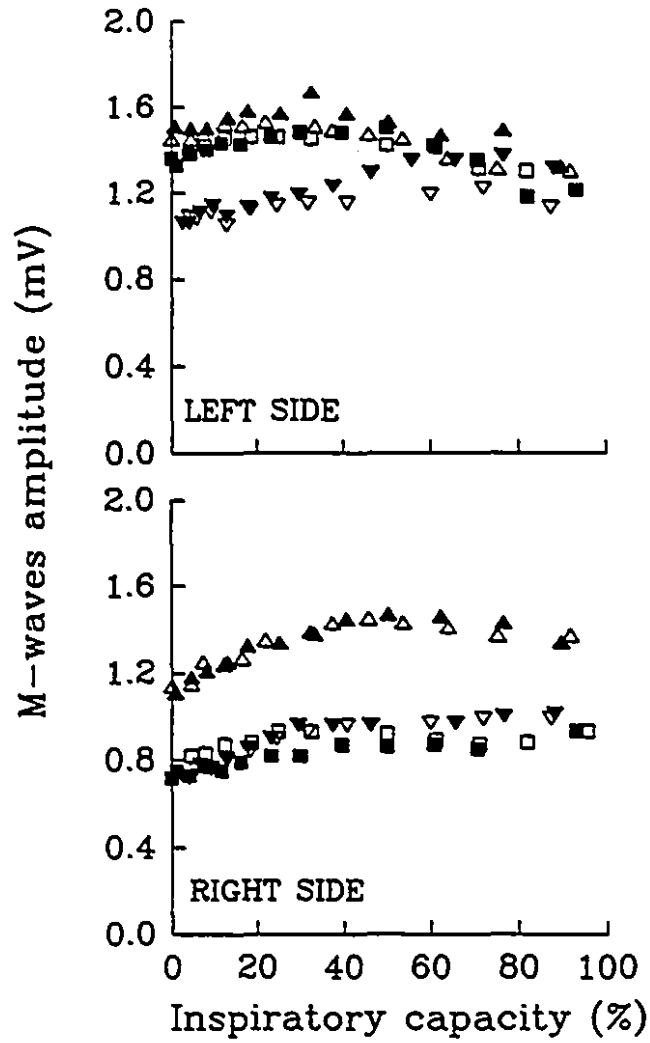

Figure 3.5: Example of M-wave amplitude (mV) as a function of lung volume (% inspiratory capacity) recorded on separate days from the fresh diaphragm of one subject. Different shapes of symbols show the results from different days and open and closed symbols with the same shape represent the results from run 1 and run 2 of the same day.

3.5 Discussion

The highly significant and reproducible relationships between Pm,T and Pdi,T that were not affected by fatigue strongly suggest that Pm,T could be substituted for Pdi,T to evaluate human diaphragmatic function noninvasively. Before discussing the implication of my results for the evaluation of diaphragm contractility, I will first consider other factors which can potentially affect this relationship.

When the respiratory muscles are relaxed and lung volume is constant, mouth pressure reflects the elastic recoil pressure of the respiratory system (18). If lung volume remains constant, changes in mouth pressure should reflect changes in pleural surface pressure. Because diaphragmatic twitches against closed airways result in essentially isovolume manoeuvres, it is understandable that I found a good relationship between Pm,T and Pes,T, and therefore between Pm,T and Pdi,T. It was previously reported (3) that, in normal subjects in sitting and lateral lying positions, the ratio of Pm to Pes measured while making inspiratory efforts against occluded airways was close to unity. Similar results were reported for inspiratory efforts performed at different lung volumes (4). Under these conditions, Pm,T should reflect pleural surface pressure changes. In the present study, however, the ratio Pm,T/Pes,T varied substantially among my subjects and was in most instances less than one (figure 3.2). Several factors could account for this finding. First, because of alveolar gas expansion during a twitch, the lung elastic recoil should increase and alveolar

pressure (and mouth pressure) swing should be smaller than pleural pressure swing. At resting lung volume at which the most negative pleural pressure was recorded, I estimate that this effect could account for a 2% difference between Pm,T and Pes,T (4). This is substantially less than the average 12% difference that I observed. Secondly, because of the transient character and short duration of the twitch, there could have been insufficient time for mouth pressure to equilibrate with alveolar pressure. However, the contraction time of single twitches of 50 to 80 ms in my subjects was 7 to 11 times longer than the estimated time constant of the airways (including the extrathoracic airways and the cheeks) of 7 to 8 ms (12). Furthermore, increasing the twitch duration by using paired stimuli instead of single shocks (figure 3.3A) had no effect on the Pm,T versus Pes,T relationship and the time of peak Pm,T and peak Pes,T concided. In my subjects, therefore, Pm,T should have been an accurate reflect of alveolar pressure swing.

A more plausible explanation for the difference observed between Pm,T and Pes,T amplitude in my subjects is that, in my experimental protocol, only the diaphragm contracted. At resting lung volume, the effect of pure diaphragmatic contraction on chest wall configuration is to increase the diameter of the lower rib cage due to the increase in abdominal pressure with an inward motion of the upper rib cage due to the fall in pleural pressure (8). The lung lengthens axially while contracting radially. Accordingly, the pressure distribution throughout the thoracic cavity is presumably non-uniform. Pleural pressure gradients with

more significant negativity over the diaphragm surface have been shown in dogs during phrenic stimulation (9). Similarly, Pes,T gradients with more significant negativity nearer to the diaphragm were noted in two of my subjects at all lung volumes (figure 3.3B). Esophageal pressure gradients have also been previously reported in humans during various voluntary manoeuvres (11). As Pes,T reflects pleural pressure change over a single point in the lung surface while Pm,T does so over the whole lung surface, in the presence of non-uniform pleural pressure, there is no particular reason to predict that Pm,T/Pes,T ratio should be in unity. With the esophageal balloon in the standard position (10 cm above the cardia) I found a significant negative correlation between Pm,T/Pes,T ratio and the height of our subjects. Under these conditions Pm,T is an even better estimate of the overall pleural surface pressure change than Pes,T measured at a single site. In addition, by contrast to the conventional Pes recordings, Pm record is virtually devoid of cardiac artifacts (figure 3.1), thereby facilitating the identification and measurement of diaphragmatic twitches, particularly when they are small such as at high lung volumes or after fatigue.

Implication of the results:

Although I did not test in the present study the relationship between Pm,T and Pes,T in patients with airway obstruction in whom equilibration between alveolar and mouth pressure requires longer time (13), available evidence suggests that upper airway compliance plays an important role in delaying pressure equilibration when

airway resistance is increased. Murciano and colleagues (17) indeed found identical values for the changes in tracheal pressure and in Pes when upper airways were bypassed by endotracheal intubation or tracheostomy. In addition, Similowski and coworkers (20) demonstrated in a group of stable patients with chronic hyperinflation that although Pm,T was smaller and lagged behind Pes,T during relaxation at FRC, Pm,T and Pes,T were in phase and equal when phrenic nerve twitches were superimposed on a gentle Meuller manoeuvre. Presumably, the negative Pm leads to a decrease in upper airway compliance so that less time is needed for mouth and alveolar pressure to equilibrate. Another possibility could be that the pleural pressure became more uniform during the Mueller manoeuvre because of smaller rib cage deformation. Based on this analysis, and provided the compliance of the upper airways can be sufficiently reduced to allow pressure equilibration within the airways (see later), the Pm,T-lung volume relationship may be helpful for monitoring diaphragmatic function even in COPD patients.

The mouth pressure measured during a maximal inspiratory effort against an occluded airway (PI,max) has been used previously to assess the strength of the inspiratory muscles (7,22). When measured this way, however, the mouth pressure swing will reflect the net pressure developed by all respiratory muscles (both inspiratory and expiratory) that are recruited by the voluntary effort. Therefore, it is not possible with this measurement to draw conclusions about diaphragmatic contractility. Furthermore, this

measurement is also dependent on the level of voluntary effort and can thus be affected by the degree of motivation and practice of the subject. Additionally, at lung volumes other than that at which the elastic recoil pressure of the respiratory system is zero, it is influenced by the system's elastic properties independent of muscle strength. Finally, a recent study has shown that $P_{I,max}$ is not a reliable measure of inspiratory muscle strength in ventilator-dependent patients (16).

The measurement of $P_{m,T}$ in response to single phrenic nerve shocks offers distinct advantages over previous measurements. First, with phrenic stimulation, the activation of the diaphragm is kept constant and maximal for all subjects, and this is easily controlled by monitoring the size of the M-waves from both hemidiaphragms and by using a current 20-30% greater than that required to produce a maximal M-wave size. The M-waves thus obtained are highly reproducible for a given recording electrode placement in a given day (figure 3.5). With this method, therefore, no cooperation from the patient is required. Secondly, it is also possible with this technique to study in isolation the contraction of the diaphragm free from any synergistic or antagonistic contribution from other muscles and without confounding influences of respiratory system's recoil. It must be stated, however, that in one additional subject whose data are not included in the present report, $P_{m,T}$-$P_{di,T}$ relationship had a positive Y-intercept, indicating the contribution of expiratory muscles to $P_{m,T}$. This resulted from the unavoidable co-stimulation of the brachial plexus

with massive movements of the arms and shoulder muscles compressing the rib cage. Clearly, the measurement of Pm,T would not be indicated in this case as it does not solely reflect diaphragmatic contractility. Nevertheless, with the present technique such contamination from brachial plexus stimulation is infrequent; that is, it was observed in 1 out of 22 consecutive subjects studied in this laboratory, and is easily discriminated by stimulating the brachial plexus without co-stimulating the phrenic nerves (as judged by the absence of diaphragmatic M-waves).

Mier and colleagues (14) reported a smaller intersubject variability in Pdi measured during a sniff (sniff Pdi) than during a twitch (Pdi,T), and that sniff Pdi discriminated diaphragmatic weakness better than Pdi,T. In this study, the measurements of sniff Pdi and of Pdi,T were obtained in different body postures so that the comparison may not be valid. Furthermore, the use of sniff Pdi as a measure of diaphragmatic strength has not yet been validated. Like other voluntary manoeuvres, this measurement is also dependent on the level of voluntary effort produced and can be affected by the recruitment of other respiratory muscles (2,10). Sniff Pdi should also depend on the degree to which upper airway resistance increases during a sniff. A decrease in sniff Pdi, therefore, does not necessarily imply that the strength of the diaphragm is also decreased. In fact, in some of the patients studied by Mier and colleagues (14) in which sniff Pdi was below the normal range, Pdi,T in response to a supramaximal phrenic stimulus was within the normal range.

Technical considerations:

Glottis closure is always a potential problem when measuring static respiratory pressures at the mouth. This problem is generally overcome by introducing a small leak in the breathing circuit (7). In the present study this problem was overcome by placing a high resistance on the expiratory side of the 2 way valve, the inspiratory side of which remained occluded. The continuous expiratory flow during the slow exhalation between TLC and FRC assured that the glottis remained open throughout. In one of my subjects who was capable of constantly keeping his glottis open at all lung volumes during relaxation against an occluded airway, the value of Pm,T, Pes,T, and Pdi,T at any given lung volume agreed within $\pm$10% with those obtained during the slow relaxation method.

For mouth pressure twitches to reflect mechanical properties of the diaphragm contracting alone, it is important to ascertain relaxation of the other respiratory muscles. With the present method, this can be achieved in 2 ways. First, the surface electrodes on each side of the chest can record the electrical activity not only from the diaphragm but also from all other underlying respiratory muscles, that is, the internal and external intercostals and some of the abdominal muscles. The absence of electrical activity other than the diaphragmatic M-waves is then an indication that the underlying respiratory muscles were relaxed at the time the diaphragmatic twitches were recorded. Secondly, reproducibility of the relaxation pressure-volume relationship of

the respiratory system (a plot of lung volume versus Pm between twitches) can be directly assessed since this is the most widely accepted criteria of respiratory muscle relaxation (1). As noted earlier, overshoots in the pressure records were frequently observed during the relaxation phase of the twitches presumably reflecting contraction of the abdominal muscles. However, these contractions should not interfere with the present analysis, because they occurred only after the peak of the twitch.

As discussed previously, the compliance of upper airways and cheeks did not appear to prevent equilibration of mouth and alveolar pressures in my subjects. The same should apply to patients with neurological or muscular disorders who have normal lungs. In patients with high airway resistance and long airway time constant, however, it may be necessary to support the cheeks externally or to use specially designed mouthpiece in order to reduce the upper airway compliance. When upper airways and cheeks are already bypassed by endotracheal intubation or tracheostomy, the measurement of tracheal pressure twitches (Ptr,T) can conveniently substitute that of PmT for noninvasive and objective evaluation of diaphragmatic function.

In the present study, Pm,T and Pdi,T were highly correlated, and both were also correlated to lung volume change. It was shown that the Pm,T versus lung volume relationships were reproducible either within or among days for a given subject. Accordingly, a sequential determination of this relationship for a given subject or patient over time may provide useful information on the

condition of the diaphragm since an upward or downward shift of this relationship over time may indicate a worsening or improvement of diaphragmatic contractility. As my results in normal volunteers showed, Pm,T during phrenic stimulation provides useful information about changes in diaphragmatic pressure generating capacity as a result of changes in lung volume or as a result of fatigue. This opens up the possibility that the noninvasive measurement of Pm,T as a function of lung volume may give useful information on diaphragmatic function in sick patients in whom measurement of Pdi is difficult.

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CHAPTER 4

FORCE-FREQUENCY RELATIONSHIPS OF IN VIVO HUMAN AND IN VITRO RAT DIAPHRAGM USING PAIRED STIMULI

In chapter 2 and 3 I have shown that changes in diaphragmatic contractility can be assessed objectively by measuring Pdi,T and Pm,T in response to phrenic nerve stimulation if lung volume is controlled as an independent variable. However, repeated measurements are required for each subject to assess changes in contractility to detect fatigue because there is always an overlap of the values between the fresh and fatigued diaphragm among subjects.

With tetanic phrenic nerve stimulation, the pressure-frequency relationship of the diaphragm can be obtained, which gives valuable information of diaphragmatic contractility not available from single twitches. Low frequency fatigue can be detected as a decrease in the ratio of Pdi at a low to that at a high stimulation frequency. Since tetanic stimulation is too painful to be applied to patients, the present chapter describes the application of phrenic nerve paired shocks as a means to evaluate diaphragmatic pressure-frequency curves and to detect low frequency fatigue.

4.1 Abstract

Supramaximal stimuli with time intervals of 100ms (10Hz) to 10ms (100Hz) were delivered in pairs to the phrenic nerves bilaterally in 5 seated normal subjects while recording transdiaphragmatic pressure swings (Pdi,s) at relaxed end expiratory lung volume with airways closed. In fresh diaphragms, Pdi,s increased between 10-20Hz and reached a plateau between 20-30Hz. Diaphragmatic fatigue decreased Pdi,s at all frequencies. Pdi,s was assumed to be the sum of 2 successive responses (T1+T2), T1 being constant at any frequency and equal to a single twitch, T2 being obtained by subtraction. I found that T2 amplitude, which was significantly reduced after fatique, was fully returned to normal after 15 min rest at high not at low stimulation frequencies. The ratio of T2 at 10Hz over 100Hz ($T2_{10/100}$) thus decreased from 1.33 ± 0.05 before fatigue to 0.97 ± 0.12 after fatigue ($p<0.025$) and to 0.81 ± 0.06 ($p<0.001$) after 15 min rest. Similar results were also obtained in isolated rat diaphragmatic strips stimulated and fatigued in vitro, from which I found a highly linear relationship ($r=0.94$, $p<0.001$) between the ratio of $T2_{10/100}$ and that of tetanic force at 10Hz over 100Hz ($P_{10/100}$). I conclude that phrenic nerve paired twitches provide similar information obtained from phrenic tetanic stimulation in terms of diaphragmatic contractility and the decrease in $T2_{10/100}$ ratio indicates diaphragm low frequency fatigue.

Key words Twitches, Transdiaphragmatic pressure, Contractile properties, Fatigue, Phrenic nerve stimulation

4.2 Introduction

Force-frequency curves contain much information about skeletal muscle contractility and are particularly useful in determining the type and severity of muscle fatigue. Skeletal muscle fatigue is conventionally divided into high and low frequency fatigue. In the former there is a selective loss of force at high stimulation frequencies and recovery from it is rapid (usually less than half an hour) while in the latter the selective loss of force occurs at low stimulation frequencies and recovery is prolonged (usually greater than one hour) (12-14). High and low frequency fatigue can be easily detected by calculating the ratio of force development at a low stimulation frequency to that at a high stimulation frequency. This ratio increases with high frequency fatigue while it decreases with low frequency fatigue (2,12-16,30,32).

Force-frequency curves of the diaphragm have been reported in humans for unilateral transcutaneous phrenic nerve stimulation using transdiaphragmatic pressure (Pdi) response as an index of force (2,31). However, the Pdi generated in response to bilateral phrenic nerve stimulation reflects more accurately the strength of diaphragmatic contractions (6,28). Furthermore, unless supramaximal stimulation is administered, data interpretation becomes difficult because one is uncertain that stimulus intensity remains constant at different frequencies. Trains of supramaximal stimuli are also very painful; the procedures can barely be tolerated unilaterally and not at all bilaterally. Single supramaximal shocks on the other hand are more easily tolerated.

In order to determine whether or not Pdi-frequency curves could be obtained from twitches rather than trains of stimuli I varied the interval between bilateral transcutaneous supramaximal phrenic nerve paired shocks from 10ms (100Hz) to 100ms (10Hz). This proved feasible in normal human subjects and much easier to achieve than tetanic stimulation. I found that the ratio of the second twitch Pdi at 10Hz expressed as a ratio of that at 100Hz ($T2_{10/100}$) is a valuable index of low frequency diaphragmatic fatigue. Further studies on rat diaphragmatic strips in vitro supported this conclusion. Thus, this chapter describes a method of measuring the Pdi-frequency relationships in vivo in humans and how it is affected by low frequency fatigue. It is potentially applicable as a clinical test.

4.3 Methods

Human study:

Subjects:

Experiments were performed on five normal male volunteers, all of whom knew the purpose of the investigation and had previous experience of being subjects for studies of respiratory mechanics.

Pressure measurements:

Esophageal pressure (Pes) and gastric pressure (Pga) were measured by the conventional esophageal and gastric balloon-catheter systems originally described by Agostoni and Rahn (1) and Milic-Emili et al. (29). All signals were recorded on tape.

Phrenic nerve stimulation:

In the present study, either single shocks or paired stimuli with interpulse intervals ranging from 10ms (100Hz) to 100ms (10Hz) were delivered to both phrenic nerves simultaneously via two synchronized constant current stimulators (Teca SC6). Figure 4.1 is a schematic illustration of the methods employed to stimulate the phrenic nerves and record the elicited diaphragmatic muscle mass action potentials (M-waves) with surface electrodes. Briefly, an electrode pad was placed just below the clavicle near the sternum on each side and served as anode. The hand-held cathodes were applied bilaterally at the phrenic motor point in the neck approximately at lower posterior edge of sternocleidomastoid muscles. The exact site and orientation of the cathodes were carefully adjusted in order to provide the "best" stimulation condition as judged by the size of the M-wave and minimal

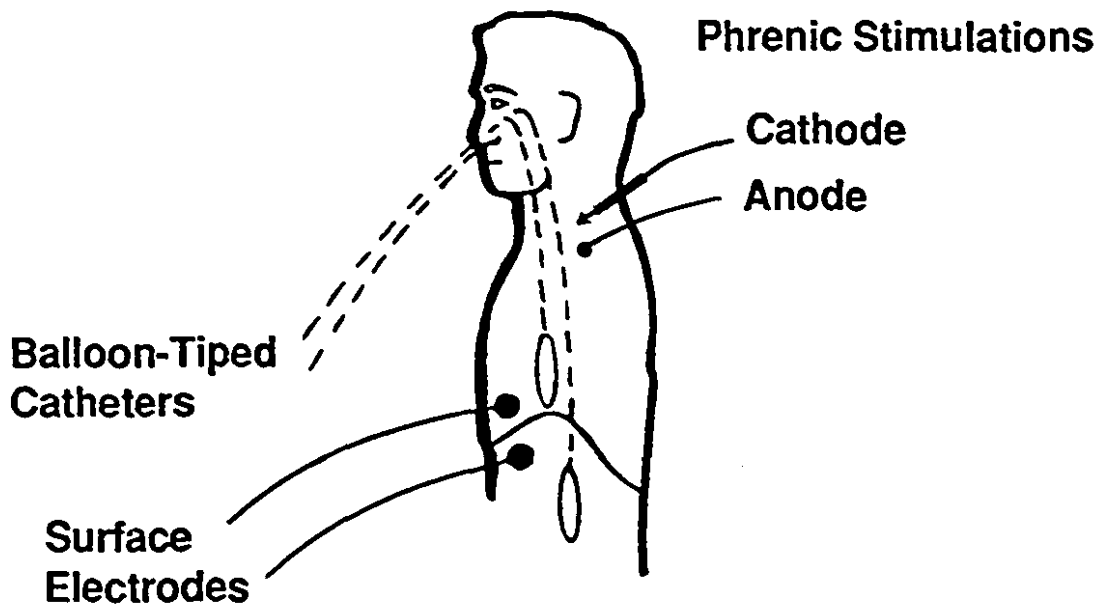

Figure 4.1: Schematic representation of the techniques to stimulate the phrenic nerves and to record the evoked diaphragmatic muscle mass action potentials (M-waves) by surface electrodes.

involvement of the brachial plexus. The stimulus current was then progressively increased until further increasing the current did not elicit any additional increase in the size of the diaphragmatic M-waves. Thereafter, the stimulus current was further increased by approximately 20%-30% and was kept constant throughout the test to ensure supramaximal stimulation. The diaphragmatic M-waves from both sides were recorded with bipolar surface electrodes. On each side, the electrodes were placed one over the 6th or 7th intercostal space between the anterior axillary line and the mid-clavicular line, the other over the adjacent costal margin. A more detailed description of the methods employed can be found elsewhere (4,5).

Experimental protocol:

The subjects were studied seated with lower rib cage and abdomen tightly bound to minimize diaphragm shortening during elicited contractions. The binding consisted of inelastic tape wrapped around the subjects from the xiphoid to the iliac crest. The measurements were done before, after the induction of diaphragm fatigue, and after 15 min rest post-fatigue. Diaphragm fatigue was induced by inspiring through an external resistance and generating a target Pdi of 60% of maximal voluntary Pdi with a duty cycle of about 0.6 as previously described (5). All measurements were made with the subjects relaxed against a closed glottis at end expiratory lung volume. For each experimental condition at each of the selected frequencies (see results below) 5-8 stimuli to the p2hrenic nerves were administered and the elicited M-waves and

pressure responses were recorded.

Animal study:

Nine strips (length 2.05-2.43 cm, weight 0.05-0.09 g) of costal diaphragm were excised from five Sprague-Dawley rats previously anesthetized with pentobarbitol sodium (50mg/Kg IP). The strips were mounted vertically between two platinum stimulating electrodes and immersed in Kreb's solution perfused with 95% O_2 and 5% CO_2 gas mixture at 25°C. This is not only the temperature optimal for maintaining stability of contractile properties (37) and of fatigure resistance (38) in vitro but also the one with a twitch duration similar to that of humans in vivo. The Kreb's solution contained 118 NaCl, 4.5 KCl, 1.5 $MgSO_4$, 1.2 KH_2PO_4, 25.5 $NaHCO_3$, 3.2 $CaCl_2$, 5.6 glucose in mM, and 0.2% curare. The strips were stimulated directly and supramaximally with 0.2 ms square wave pulses to contract isometrically with single and paired shocks as in the human study. In addition, the strips were also stimulated with 4 pulses and trains of pulses (duration: 500 ms) from 10Hz to 100Hz. Six strips were fatigued by supramaximal twitch stimulation (0.2 ms square wave pulses and 1.5 pulses per second) until the twitch force decreased to about 50% of the control value. After fatigue, and also following 15 and 30 min recovery, single twitches, paired stimuli and tetanic stimulation at 10Hz and 100Hz were repeated. All measurements were made with the muscle strips held at the optimal length for single twitch stimulation (26). The force output was recorded by a Kulite force transducer (Model: LOAD CELL BG-100 GRAMS) and expressed conventioanlly as newton per

square centimeter (N/cm^2) (33).

Data management:

For human studies, the pressure and M-wave signals recorded on tape were fed through a 12 bit analog to digital converter to a computer for data analysis. The sampling rate was set at 500Hz for pressure signals and 2000Hz for M-wave signals. Pdi was obtained by digitally subtracting Pes from Pga. The electrical and pressure responses to the phrenic nerve shocks were isolated over an appropriate time window (usually 500 ms) and then averaged by the computer. At least five responses were averaged at each frequency under each condition. Peak amplitude of the averaged signals was measured relative to the immediately preceding baseline and referred to as Pdi,s. The force recorded in animal studies was directly fed to the computer through the analog to digital converter. Five single twitches were averaged in each condition. The analysis was the same as in human study.

In this paper, a single response refers to the electrical or mechanical response to a single shock, i.e. an ordinary twitch, whereas a paired response refers to the response to paired stimuli. A paired response in turn is assumed to be the sum of two successive responses, the first of which is constant for a given condition and equal to the single response, the second response being the residual between the paired and the first responses. Based on assumption, the second M-waves (M2) and the second twitches (T2) (Pdi in human study or tension in animal study) were obtained by digital subtraction on computer.

Statistical assessment was performed using paired "t" test, the analysis of variance (ANOVA), and standard linear regression techniques as appropriate. The criterion for statistical significance was $p<0.05$. Values reported in text and given in figures are Means$\pm$1SE.

4.4 Results

Human study:

Electrical responses:

Representative M-wave responses from one subject at 4 different frequencies are shown in figure 4.2, upper panel. The middle panel of figure 4.2 shows the corresponding second M-waves (M2) obtained by subtraction. The amplitude of M2 progressively decreased with increasing frequency (ANOVA $p<0.001$) so that at 100Hz, it was 78% of a single M-wave's amplitude. However, this change was not influenced by fatigue and recovery. The first M-wave (M1) was independent of frequency but significantly different as a result of fatigue and recovery compared to fresh condition (ANOVA $p<0.001$). However, since the mean amplitudes of M1 in the 3 conditions were, in fact, close (Fresh: 98.6 ± 0.7%, fatigue: 105.6 ± 1.6%, and recovery: 93.6 ± 1.6%) to that of a fresh single M-wave, the differences were considered most probably not to be physiologically meaningful.

Mechanical responses:

Examples of Pdi responses to paired stimuli at 4 tested frequencies are shown for one subject in the lower panel of figure 4.2. The first (T1) and second responses (T2) are also indicated. The summation of Pdi responses to each of the two successive stimuli is clearly seen at 10Hz but less clearly at 20Hz and at higher frequencies. In 3 of the subjects, repeated determinations of Pdi,s-frequency relationships were made on 2-3 separate days over a period of 1 to 20 weeks (figure 4.3). No significant difference

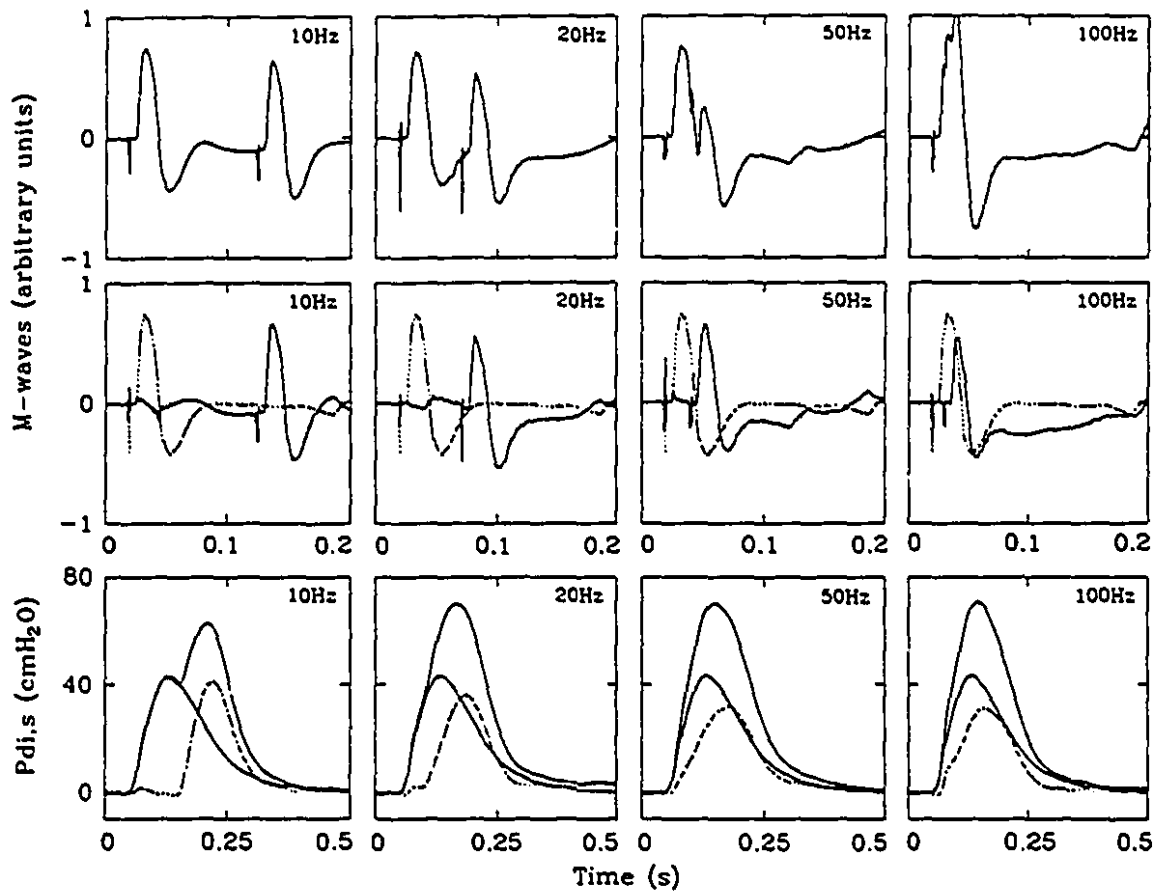

Figure 4.2: Upper panel, records of M-waves from left hemidiaphragm of one subject in response to paired shocks at 4 stimulation frequencies. Middle panel, records of the second M-waves (M2) obtained after subatraction at each corresponding frequency. A M-wave response to a single shock is also shown at each frequency (dotted tracings). Lower Panel, representative Pdi reponses to single shock (lower solid curve) and to paired shocks (upper solid curve) are shown for one subject. Each panel is superimposed by the second twitch response (T2) (dotted curve) obtained by subtraction at that frequency (see methods).

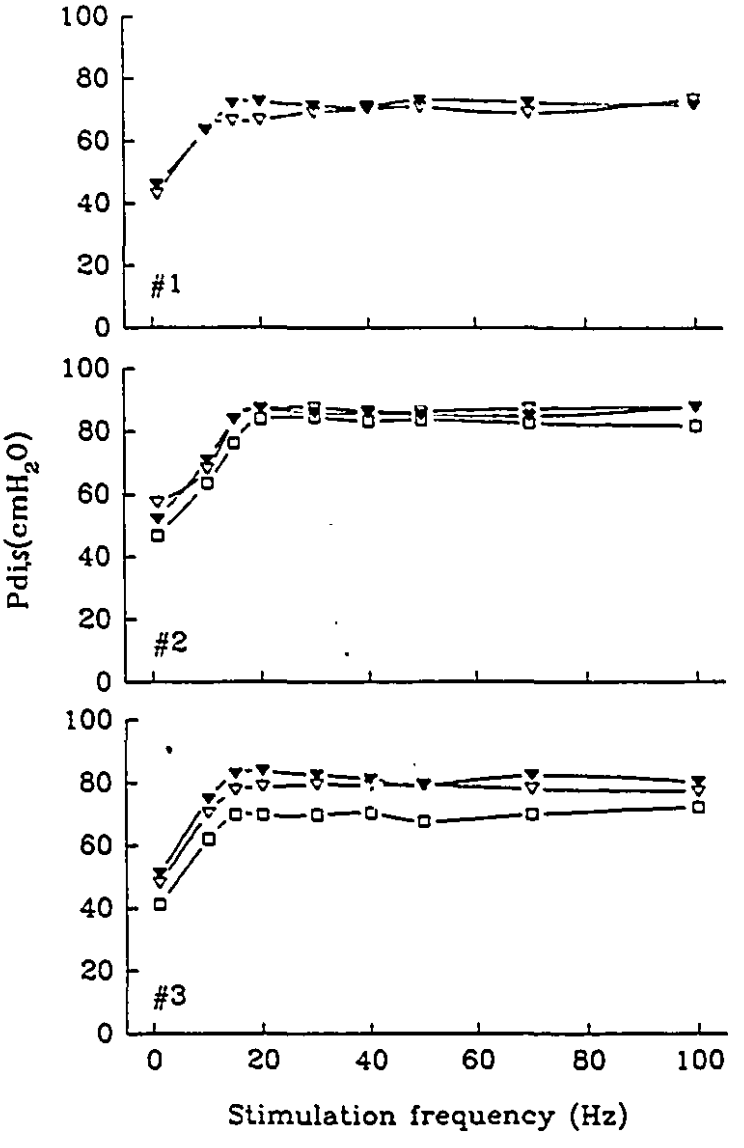

Figure 4.3: Pdi,s-frequency curves in response to bilateral phrenic nerve paired stimuli from 3 subjects when the test was repeated on separate days over 1 to 20 weeks (different symbols).

was found on repeated trials (ANOVA $p < 0.8$), the small between day variability being presumably accounted for by slight differences in the degree of abdominal binding.

As shown in figure 4.4a, Pdi,s increased between 10Hz and 20Hz. Further increase in stimulation frequency did not result in greater Pdi,s. Pdi,s decreased at all frequencies after fatigue (ANOVA $p<0.0001$) and recovered only minimally after 15 min rest. The ratio of Pdi,s at 10Hz over that at 100Hz ($Pdi,s_{10/100}$) decreased from 0.88 ± 0.03 before fatigue to 0.76 ± 0.05 immediately after the fatigue run ($p<0.05$) and to 0.68 ± 0.05 after 15 min rest ($p<0.01$).

Figure 4.4b shows that in the fresh state, the amplitude of T2 decreased with increasing stimulation frequency. The shape of this relationship was markedly affected by fatigue: Fatigue preferentially depressed T2 amplitude at low stimulation frequencies, which recovered slowly. By contrast, the small decay of T2 at high frequencies recovered quickly. Because of the preferential fall of T2 at low frequencies and its quick recovery at high frequencies, the amplitude ratio of T2 at 10Hz over 100Hz ($T2_{10/100}$) decreased significantly from a control value of 1.33 ± 0.05 to 0.97 ± 0.12 ($p<0.025$) immediately after the fatigue run and to 0.81 ± 0.06 ($p<0.001$) after 15 min rest (figure 4.4c).

Animal study:

The contractile properties obtained from rat diaphragm strips in fresh condition (table 4.1) were comparable to previously published values at 25°C (27,34). The force-frequency curves of fresh diaphragm strips from paired shocks, from 4 pulses, and from

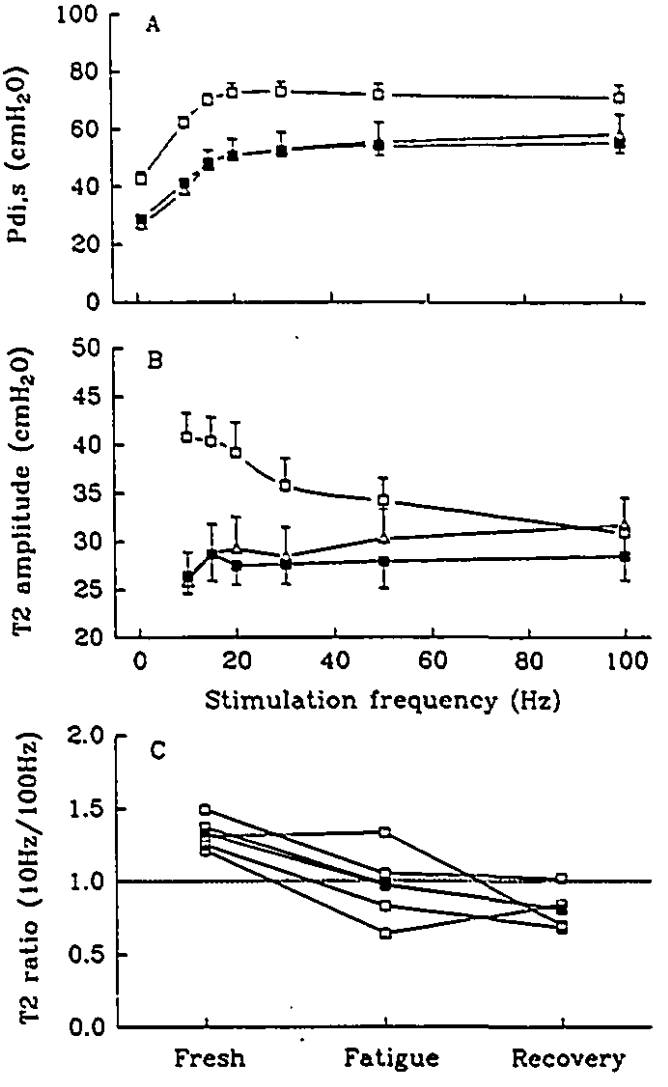

Figure 4.4: a: Group mean Pdi,s-frequency curves of the diaphragm in response to bilateral phrenic nerve paired stimuli before (open squares), after fatigue (closed squares), and after 15 min recovery (open triangles). b: Group mean amplitude of the second twitch (T2) plotted as a function of stimulation frequency before (open squares), after fatigue (closed squares), and after 15 min recovery (open triangles). c: Ratio of T2 at 10Hz over that at 100Hz ($T2_{10/100}$) before, after fatigue, and after 15 min recovery. Open squares are for individual data and closed squares for group mean.

train of pulses are shown in figure 4.5. In all cases the force increased with increasing stimulation frequency. At any frequency the force also increased with increasing number of stimuli especially at high frequencies. The frequency at which the force became maximal also increased with increasing number of stimuli. Consistent with the human in vivo data of this study, the amplitude of T2 calculated in the same manner from paired responses progressively decreased with increasing frequency of stimulation.

Table 4.1 Contractile properties of rat diaphragm strips

n	Pt(N/cm^2)	CT(ms)	1/2RT(ms)	Po(N/cm^2)	Pt/Po
9	10.49±0.41	39.4±1.0	47.0±1.8	23.83±1.01	0.44±0.01

Values are mean±SE (n=9). Pt: single twitch tension, CT: contraction time, 1/2RT: half relaxation time, Po: maximal isometric tension (recorded at 100Hz and at optimal length).

Table 4.2 Effect of fatigue and recovery on rat diaphragmatic strips

			Recovery	
(n=6)	Fresh	Fatigue	15 min	30 min
Pt	10.05±0.29	4.87±0.17‖	5.85±0.21‖	6.51±0.22§
Pa_{10}	11.73±0.35	5.95±0.28‖	6.94±0.37§	7.76±0.41§
Pa_{100}	15.17±0.47	8.43±0.29‖	10.30±0.28§	11.59±0.24‡
P_{10}	12.55±0.26	6.78±0.33‖	7.33±0.44‖	8.16±0.50‖
P_{100}	23.38±0.88	14.89±0.41§	18.48±0.43‡	20.60±0.57*

Values are mean±SE (n=6) and expressed as N/cm^2. P_t: single twitch, Pa_{10}: paired twitches at 10Hz, Pa_{100}: paired twitches at 100Hz, P_{10}: tetanic force at 10Hz, P_{100}: tetanic force at 100Hz. Compared with fresh state, * $p < 0.05$; ‡ $p < 0.01$; § $p < 0.001$; ‖ $p < 0.0001$.

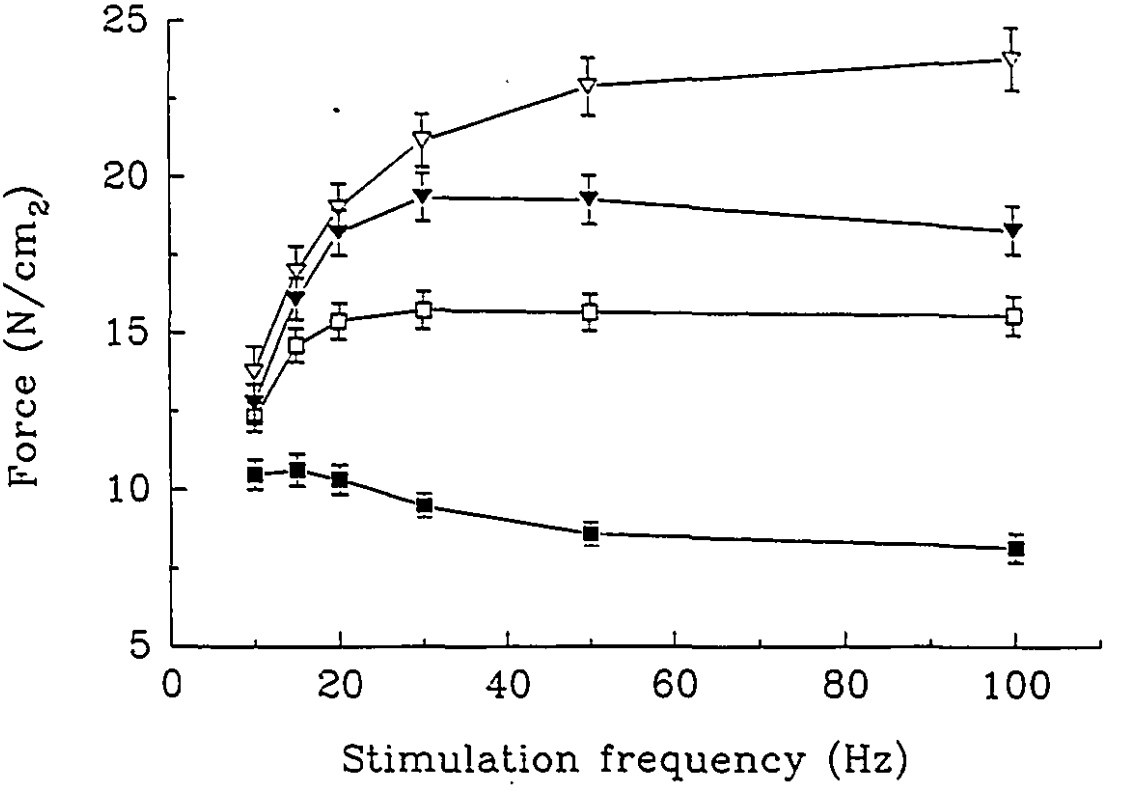

Figure 4.5: Force-frequency relationships of fresh rat diaphragm strips with tetanic stimulation (open triangles), four shocks (closed triangles), paired shocks (open squares), and T2 response to paired shocks (closed squares).

The effects of fatigue and recovery on the force of single twitch, paired shocks, and tetanic stimulation at 10Hz and 100Hz are summarized in table 4.2. All responses decreased with fatigue though more at low than at high stimulation frequencies and all showed the tendency to recover during the 30 min recovery period. As shown in figure 4.6, $T2_{10/100}$ and tetanic force ratio at 10Hz over that at 100Hz ($P_{10/100}$) changed in a similar way with fatigue and recovery ($p<0.005$). When $T2_{10/100}$ and $P_{10/100}$ are plotted one against another under all conditions, a highly significant correlation ($r=0.94$, $p<0.001$) was found (figure 4.7). Fatigue and subsequent recoveries shifted this relationship towards lower values.

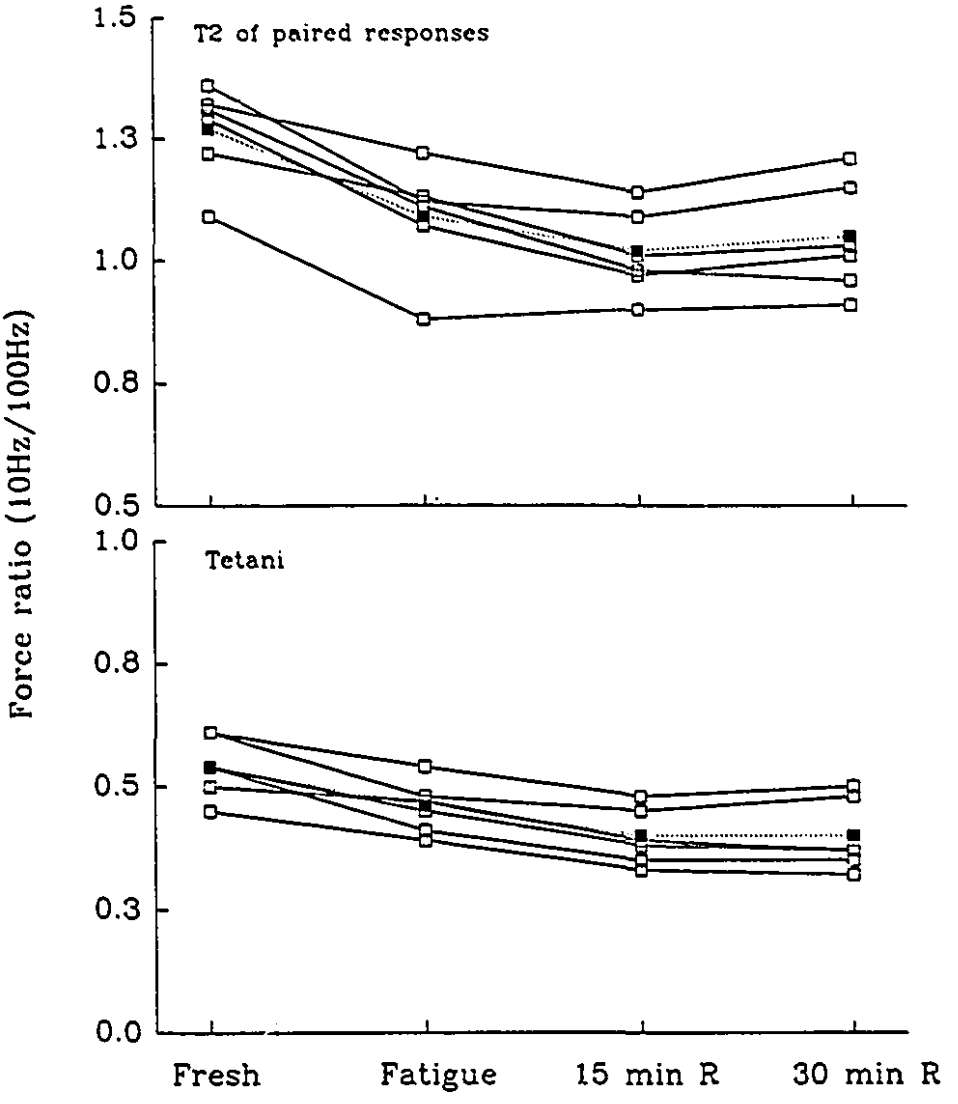

Figure 4.6: For the rat diaphragm, ratio of T2 (upper panel) and of tetanus (lower panel) at 10Hz over that at 100Hz before, after fatigue, and following recoveries. Conventions are the same as those in figure 4.4c.

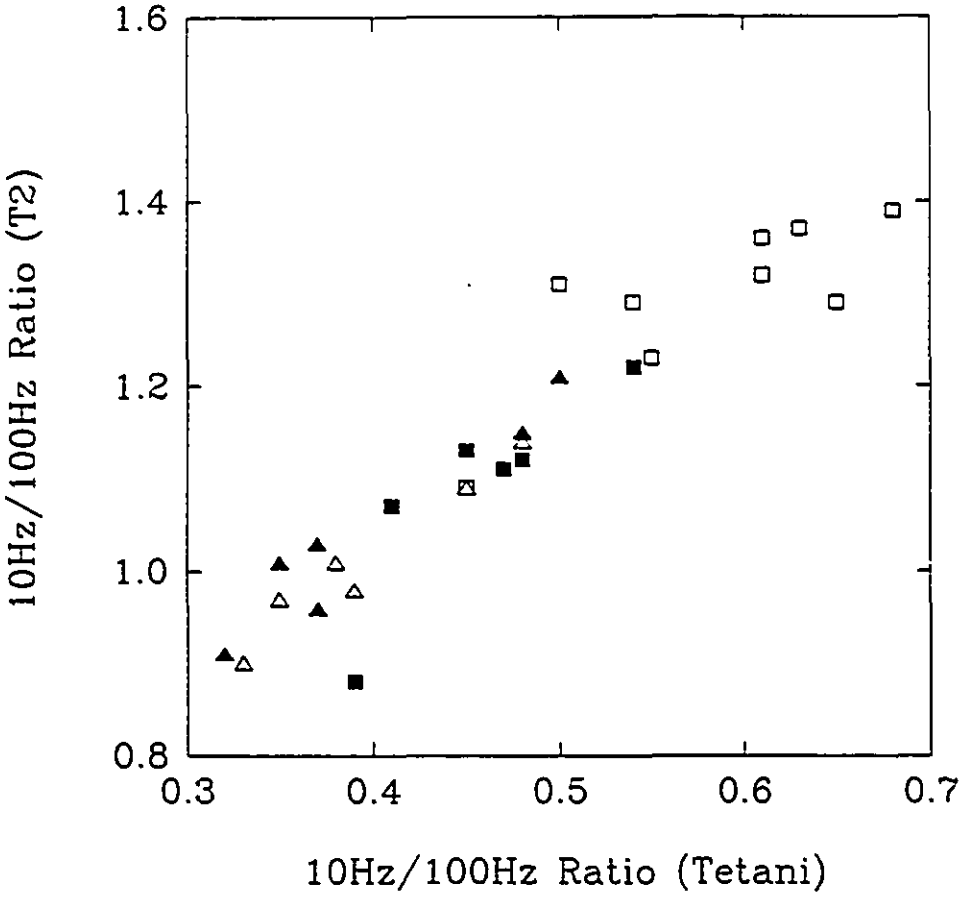

Figure 4.7: For the rat diaphragm, ratio of T2 plotted as a function of ratio of tetaus at 10Hz over 100Hz before (open squares), after fatigue (closed squares), after 15 min recovery (open triangles), and after 30 min recovery (closed triangles).

4.5 Discussion

Force-frequency relationship of the diaphragm:

As shown for the in vitro rat diaphragm preparations, the force-frequency relationship differs depending on the number of impulses in the train (figure 4.5). Comparable differences can be found for the human in vivo diaphragm by comparing the Pdi-frequency relationships obtained here using paired stimuli with those previously reported using trains of tetanic stimulation (2,6,31). Compared with single twitches, paired stimuli elicited additional Pdi or force which was expressed by T2. With both in vitro rat and in vivo human diaphragm preparations the T2 amplitude decreased progressively with increasing frequency, as could be expected based on the characteristic shape of the force-frequency relationships for both muscles (2,19,31). Without this decrease, the Pdi or force during a long tetanus would not tend towards a finite maximal value as frequency increases.

Effect of fatigue and recovery:

Peripheral fatigue of skeletal muscles has been conventionally subdivided into low and high frequency fatigue components based on the different rate at which the force recovers when measured in response to low (10-20Hz) or high (50-100Hz) stimulation frequencies (12-14). Both types of fatigue have been documented previously in normal human diaphragm in vivo using unilateral phrenic nerve tetanic stimulation (2,31). Low frequency fatigue of the respiratory muscles has been thought to have important clinical significance for the following reasons. First, there is always a

preferential loss of force in the low frequency range when skeletal muscles do intense exercise (2,31,32). Secondly, low frequency fatigue generally lasts for many hours after the cessation of the fatiguing task (2,13,32). Thirdly, the firing frequency of skeletal muscles for daily activities is within the low frequency range (12,22). Finally, increasing severity of low frequency fatigue is associated with progressively decreased endurance time (15).

Low frequency fatigue of the diaphragm, like that of other skeletal muscles, can be identified by calculating the ratio of Pdi generated at a low frequency (generally 20Hz) over that generated at a high frequency (50 or 100Hz) (2,31,32). This ratio normally is quite stable, but decreases significantly following a diaphragmatic fatiguing task (30,31,36). The present study using bilateral phrenic nerve paired shocks demonstrated that, as expected, fatigue decreased Pdi,s at all stimulation frequencies. There was also a less pronounced but statistically significant decrease in $Pdi,s_{10/100}$ with fatigue. However, in contrast to previous studies, the Pdi response to high frequency stimuli only recovered a little after 15 min rest (figure 4.4a).

These differences are best explained when considering the Pdi response to paired stimuli as the sum of two successive responses (T1+T2), the first being equal to a single twitch and independent of the stimulation frequency. Low frequency fatigue leads to a reduction in twitch amplitude (21,23). The reduction in the size of the single twitch and hence of T1 in our subjects due to the presence of low frequency fatigue would have a carry-over effect at

all stimulation frequencies, thus explaining both the relatively smaller decrease of $Pdi,s_{10/100}$ with fatigue and the persistent decline of the paired responses even at high frequencies after 15 min rest. This interpretation is supported by the progressively increasing recovery of T2 with increasing stimulation frequency after 15 min rest in human study (figure 4.4b). Because of this characteristic of paired twitches, $Pdi,s_{10/100}$ would be less sensitive to reflect low frequency fatigue compared with tetanic stimulation from which the carry-over effects of the first twitch would be very small. However, this in turn raises the possibility that the clearly distinct rates of recovery of T2 amplitude at low and high frequencies carries the same information as the ratio of Pdi at 20Hz to that at 50Hz or 100Hz obtained from tetanic phrenic stimulation. Both may be of equal value in demonstrating low frequency diaphragmatic fatigue in humans. My results from in vitro rat diaphragm strips show that this is indeed the case (figure 4.6 and 4.7). What's more, the bilateral T2 measurements at 10 and 100Hz are tolerable, whereas bilateral supramaximal tetanus at these frequencies are not (6).

As shown in figure 4.4c, there was a considerable decrease in $T2_{10/100}$ ratio with fatigue and after 15 min recovery. The only exception was from one subject whose $T2_{10/100}$ did not change immediately after fatigue run, which could be explained at least in part by twitch potentiation following an intense muscular work in performing the fatiguing task (8). For this subject, $T2_{10/100}$ ratio decreased to 50% of the post-fatigue value after 15 min rest. This

strongly suggests that the decrease of $T2_{10/100}$ ratio can be masked by twitch potentiation immediately following the cessation of a fatiguing task in some individuals. By simply repeating the measurements after 15 min, not only makes this test more sensitive because twitch potentiation is no longer a confounding variable, but (in the limited number of subjects we studied) also provides a value in which there is no overlap between fresh and fatigued conditions. My data suggest that a single value of $T2_{10/100}$ smaller than 1.1 indicates the presence of low frequency fatigue. Whether or not a single value of $T2_{10/100}$ can be used to diagnose low frequency fatigue needs further investigation. In this connection, a number of indices including maximal inspiratory pressure (7), single Pdi twitch amplitude (3), power spectrum analysis (20), and relaxation characteristics of voluntary and stimulated Pdi (17) have been used to evaluate human diaphragm fatigue and have been suggested as clinically useful diagnostic tests. Unfortunately, interpretation of all of these indices requires at least 2 measurements over time because there is considerable overlap of the values from the fresh to the fatigued state so that a comparison of the fatigued with the fresh or recovered value is necessary in order to have diagnostic significance, which makes these measurements inconvenient and time consuming. An index of low frequency fatigue which does not need repeated determinations is therefore highly desirable for clinical purpose. The $T2_{10/100}$ ratio may meet this criterion.

That I found a considerable overlap of $T2_{10/100}$ ratio between

fresh and fatigued in vitro rat diaphragm strips is not surprising since many differences are believed to exist between human in vivo and rat in vitro studies. As has been suggested by many authors (18,24,35), the most important differences may be the fatigue protocols of in vitro and in vivo studies and their different rate of recovery particularly at high frequencies. Thus the fact that there are overlap between the fresh and fatigued values in rats does not influence my hope that a single measurement of $T2_{10/100}$ in humans may be diagnostically useful.

Technical considerations:

Paired stimuli has been used previously in limb muscles to study the correlation between twitch contraction and force summation (9), the active state (10,25), and the isometric contraction of single motor unit (11). To my knowledge, however, this has not been used previously in the study of respiratory muscles. In terms of reproducibility, it has been shown that diaphragmatic single twitches in response to supramaximal phrenic nerve stimulation are highly reproducible for a given subject when the determinations are made on separate days (4,40). I now showed in the present study that this is also the case for diaphragmatic paired twitches (figure 4.3).

A requirement of proper paired twitch measurement is that the abdomen and lower rib cage need to be restricted to reduce the shortening of the diaphragm (4,39) as much as possible. For clinical applications, this procedure needs to be further standardized.

In summary, phrenic nerve paired shocks provide similar information as with tetanic phrenic stimulation in terms of diaphragmatic contractility and function. The technique can be done bilaterally with reproducibility and is well tolerated by normal subjects so that it is more applicable than tetanic phrenic nerve stimulation to study diaphragmatic contractility. My results showed that the $T2_{10/100}$ ratio is a valuable index for assessing diaphragmatic low frequency fatigue, a finding well supported by in vitro rat diaphragmatic strip studies. However, further evaluation of the technique is needed before it can be recommended for clinical use due to the relatively small number of subjects tested in the present study.

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CHAPTER 5

EFFECT OF HUMAN DIAPHRAGMATIC FATIGUE ON RESPIRATORY MUSCLE CONTROL AND VENTILATORY RESPONSE DURING CO_2 REBREATHING

Many investigators have hypothesized that respiratory muscle fatigue may be an important cause of ventilatory failure in patients. With this hypothesis two important questions need to be answered: [1] Does respiratory muscle fatigue occur in patients? [2] If it does, how does it affect ventilation and its control? My work presented in chapters 2 through 4 deals with phrenic nerve stimulation and diaphragmatic twitch measurement for the purpose of developing this as a clinical diagnostic procedure to test whether or not respiratory muscle fatigue exists in patients, and therefore to answer the first question. Although much is known about respiratory muscle fatigue and ventilatory control, the possible interactions between the two have not been systematically investigated. Understanding of these interactions could provide the basis to answer the second question, because fatigue may alter ventilatory control by altering pressures and degree of shortening of respiratory muscle recruitment so as to alter ventilation, respiratory frequency, tidal volume, and the pattern of the respiratory pressure swings. I therefore designed the experiments presented in chapter 5 and chapter 6 to assess if inspiratory muscle fatigue affects ventilation and its control. First, I tested the effects of diaphragmatic fatigue because the diaphragm is the major inspiratory muscle.

5.1 Abstract

I studied the influence of diaphragmatic fatigue on the control of ventilation and respiratory muscle recruitment, in 6 normal seated subjects. CO_2 was rebreathed before and after diaphragmatic fatigue induced by breathing against an inspiratory resistance requiring 60%-70% maximal transdiaphragmatic pressure with each breath until exhaustion. Diaphragmatic fatigue was confirmed by phrenic nerve stimulation. It was found that for a given level of end-tidal CO_2 partial pressure ($P_{ET}CO_2$) none of the ventilatory parameters I measured including tidal volume (V_T), breathing frequency (f), minute ventilation ($\dot{V}_E$), Ti/Ttot, and mean inspiratory flow (V_T/Ti) changed (ANOVA) following diaphragmatic fatigue. During CO_2 rebreathing, ΔPes was the same while ΔPga and ΔPdi significantly decreased after diaphragmatic fatigue for a given $P_{ET}CO_2$. The slope of transpulmonary pressure (P_L)-Pga relationship determined at zero-flow points at the end of expiration and inspiration, which increased significantly with increasing $P_{ET}CO_2$, was further increased at any given $P_{ET}CO_2$ after diaphragmatic fatigue. End-expiratory P_L progressively decreased and end-expiratory Pga progressively increased with increasing $P_{ET}CO_2$ by the same magnitude before and after diaphragmatic fatigue. I conclude that diaphragmatic fatigue induces proportionately greater recruitment of inspiratory rib cage muscles than of the diaphragm, which results in the preservation of ventilatory response to CO_2 despite impaired diaphragmatic contractility. Diaphragmatic fatigue has little effect on the progressive

activation of expiratory muscles stimulated by CO_2.

KEY WORDS: intercostal muscles; expiratory muscles; respiratory muscle coordination; recruitment; transdiaphragmatic pressure

5.2 Introduction

It has frequently been postulated that inspiratory muscle fatigue is a cause of acute hypercapnic respiratory failure (37,38). Testing this hypothesis has been difficult because of the lack of a specific and objective test to detect inspiratory muscle fatigue (35). The lack of evidence either for or against the hypothesis has led to a state of confusion because it is unclear whether, in the face of a fatiguing load, the ventilatory control system operates to protect against fatigue by decreasing inspiratory muscle power output and tension development at the expense of alveolar hypoventilation and hypercapnia or whether the muscles are driven so that fatigue develops and alveolar hypoventilation results. An additional possibility is that there is an inherent abnormality of the ventilatory control system so that hypercapnia may result even under conditions when the load is insufficient to cause fatigue.

In order to clarify the events which lead to acute hypercapnic respiratory failure, the relationship between inspiratory muscle fatigue and the chemical control of breathing must be understood, as it is highly likely that chemical control of ventilation is affected by inspiratory muscle fatigue. Fatigue induces rapid shallow breathing both in patients (10) and in normal subjects (16) under eupnoeic conditions, which in patients appears to result in hypercapnia (10). Nevertheless, the influence of fatigue on respiratory muscle recruitment and control of ventilation has not been systematically investigated. The purpose of the present

work was to quantify the effect of diaphragmatic fatigue on respiratory muscle recruitment and the ventilatory response to inhaled CO_2.

5.3 Methods

Subjects:

Six healthy male subjects (age: 40.7±4.6) participated in the present study. All of them were members of laboratory staff but three of them were naive with regard to the purpose of the study. The protocol was approved by the Ethics Committee of the Montreal Chest Hospital and informed consent was obtained from all the subjects.

Measurements:

Each subject was comfortably seated in an armchair and breathed through a two way valve connected by a mouthpiece with a noseclip. One port of the two way valve was connected to the rebreathing bag and the other port to room air. Esophageal pressure (Pes) and gastric pressure (Pga) were measured relative to atmospheric pressure by conventional balloon-catheter systems (32) attached separately to two differential pressure transducers (Honeywell 8604). Transdiaphragmatic pressure (Pdi) was obtained by electrical subtraction of Pes from Pga. Flow was measured by a pneumotachograph (Fleisch #2) near the mouthpiece and lung volume change was obtained by integrating the flow signal. End-tidal CO_2 concentration and partial pressure ($P_{ET}CO_2$) was continuously monitored at the mouthpiece using a carbon dioxide analyzer system (Ametek CD-3A) which was calibrated before each test with standard and chemically analyzed gas mixtures.

Procedures:

CO_2 rebreathing: CO_2 rebreathing was performed using the method

described by Read (34). The subjects breathed through a rebreathing bag with 6-8 liters of mixed gas containing 7% CO_2 balanced with O_2. The procedure was terminated when end-tidal CO_2 concentration reached about 8% and this generally took 3-4 min.

Induction of diaphragmatic fatigue: Diaphragmatic fatigue was induced by having the subjects inspire through a high inspiratory resistance. With each inspiration, the subjects were asked to target a Pdi which was 60%-70% of each subject's maximal Pdi (Pdi,max) and maintained it as a square wave throughout inspiration with a duty cycle of 0.6. The target Pdi was continuously displayed on a storage oscilloscope. Expiration was unloaded. Diaphragmatic fatigue was considered to have occurred when the subjects were unable to reach and maintain the target Pdi for more than 3 consecutive breath and/or the subjects felt that they could not continue and came off the mouthpiece.

Diaphragmatic fatigue was evaluated by monitoring the amplitude of Pdi twitch (Pdi,T) response to bilateral supramaximal transcutaneous phrenic nerve single shocks before and after the fatigue task. The detailed description of this technique can be found elsewhere (5,6).

Experimental protocol:

At the start of each test, 5-10 Pdi,Ts were recorded with the subjects relaxed at end-expiratory lung volume and their glottis closed. The subjects then went on the mouthpiece and breathed room air spontaneously for a few minutes until all the measured variables stabilized. The valve was then turned connecting the

subjects to the CO_2 rebreathing bag. This was done by an operator without the subjects' knowledge and the CO_2 rebreathing test began. This test generally took 3-4 min. Following termination of the control CO_2 rebreathing test with at least 10 min rest thereafter, the subjects performed the diaphragmatic fatiguing task as already mentioned. Afterwards, the same Pdi,T measurement and CO_2 rebreathing test were repeated.

Data processing and analysis:

The pressures, flow, volume, and end tidal CO_2 concentration were recorded on an eight channel strip chart recorder (Gould). All the signals but volume were also recorded on a magnetic tape recorder (Vetter, modal DI) for further analysis. These signals were then passed through a 12 bit analog-to-digital converter and digitized at 500Hz for twitch signals and at 200Hz for CO_2 rebreathing signals. They were analyzed by a computer using Anadat-Labdat software package (J.H.T. Bates, Meakins-Christie Laboratories). Pdi and volume signals were generated by the computer by subtracting Pes from Pga and integrating flow, respectively. The volume was corrected to B.T.P.S. conditions. Pdi,T's were averaged and the peak amplitude was measured relative to the immediately proceeding baseline. CO_2 rebreathing signals were analyzed on a breath-to-breath basis. Pes, Pga, and Pdi signals were measured both as a peak amplitude relative to the immediately proceeding baseline (ΔPes, ΔPga, and ΔPdi, respectively), and as an absolute value at the start and end of each inspiration (points of no flow) referred to as Pes, Pga, and

Pdi, respectively.

The response to CO_2 was analyzed both in terms of ventilation and its parameters and in terms of the output of the diaphragm, the inspiratory muscles of the rib cage, and the expiratory muscles. The ventilatory parameters measured were minute ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f), duty cycle (Ti/Ttot, where Ti = inspiratory time and Ttot = total respiratory cycle time), and mean inspiratory flow (V_T/Ti). The diaphragmatic response to CO_2 was assessed by plotting Pdi against $P_{ET}CO_2$; the contributions of the inspiratory rib cage muscles were inferred from tidal ΔPes relative to ΔPga and ΔPdi as a function of $P_{ET}CO_2$ and from the slope of the transpulmonary pressure (P_L)-Pga relationship between zero flow points at beginning and end inspiration (27). Expiratory muscle recruitment was assessed by measuring decrease in P_L and increase in Pga at end-expiration.

All the data are expressed as mean±s.e.. $P<0.05$ was considered as statistically significant. Pdi,T was compared before and after fatigue with paired t test. CO_2 rebreathing data was analyzed using two way ANOVA analysis.

5.4 Results

Twitch responses of the diaphragm to phrenic nerve stimulation are shown in table 5.1. On average, diaphragmatic fatigue produced a 32.1% depression of Pdi,T amplitude but left Pes,T/Pdi,T ratio unaltered.

Table 5.1. Diaphragmatic twitch response.

Subject	Pdi,T		Pes,T/Pdi,T	
	Fresh	Fatigue	Fresh	Fatigue
1	17.1	11.2	0.44	0.52
2	32.8	23.5	0.49	0.50
3	23.4	14.8	0.62	0.60
4	23.8	15.7	0.56	0.53
5	29.9	21.5	0.47	0.51
6	17.7	11.6	0.56	0.59
Mean	24.1	16.4	0.52	0.54
SE	2.6	2.1	0.03	0.02

Pdi,T: twitch transdiaphragmatic pressure amplitude; Pes,T: twitch esophageal pressure amplitude. The data shown for each subject were the average of at least 5 twitches before and after fatiguing run obtained at FRC during relaxation with closed airways and expressed as cmH_2O.

The breathing pattern data during CO_2 rebreathing before and after diaphragmatic fatigue were shown in figure 5.1, in which V_T,

f, $\dot{V}_E$, Ti/Ttot, V_T/Ti are plotted as a function of $P_{ET}CO_2$. After diaphragmatic fatigue, one subject increased $\dot{V}_E$ mainly by increased f and another decreased $\dot{V}_E$ mainly by decreased V_T for a given $P_{ET}CO_2$, the remaining 4 subjects did not show any consistent change. Consequently, as a group, none of the parameters of breathing pattern examined was significantly altered after diaphragmatic fatigue, the respective P value being shown in each corresponding panel of figure 5.1.

A representative recording of Pes and Pga changes in response to CO_2 in one subject before and after diaphragmatic fatigue is shown in figure 5.2. With increasing $P_{ET}CO_2$ before diaphragmatic fatigue, the amplitude of Pes swing increases reflecting the V_T response to CO_2. The difference between Pga and Pes or Pdi also increases indicating increasing recruitment of the diaphragm. With increasing $P_{ET}CO_2$ there is also a progressive rise in end-expiratory Pga and Pes indicating progressive expiratory muscle recruitment and decrease in end-expiratory lung volume. With high CO_2 drive both Pga and Pes fall abruptly by nearly equal amounts at the onset of inspiration indicating a relaxation of expiratory muscles and passive inspiration accomplished by the elastic recoil pressure of the respiratory system. Subsequently, Pes continues to decrease at a slower rate while Pga increases, signalling the onset of active inspiration and diaphragmatic recruitment. At end-inspiration Pga falls, Pes rises indicating diaphragmatic relaxation. This is followed by nearly equal increases in Pga and Pes as a result of expiratory muscle recruitment.

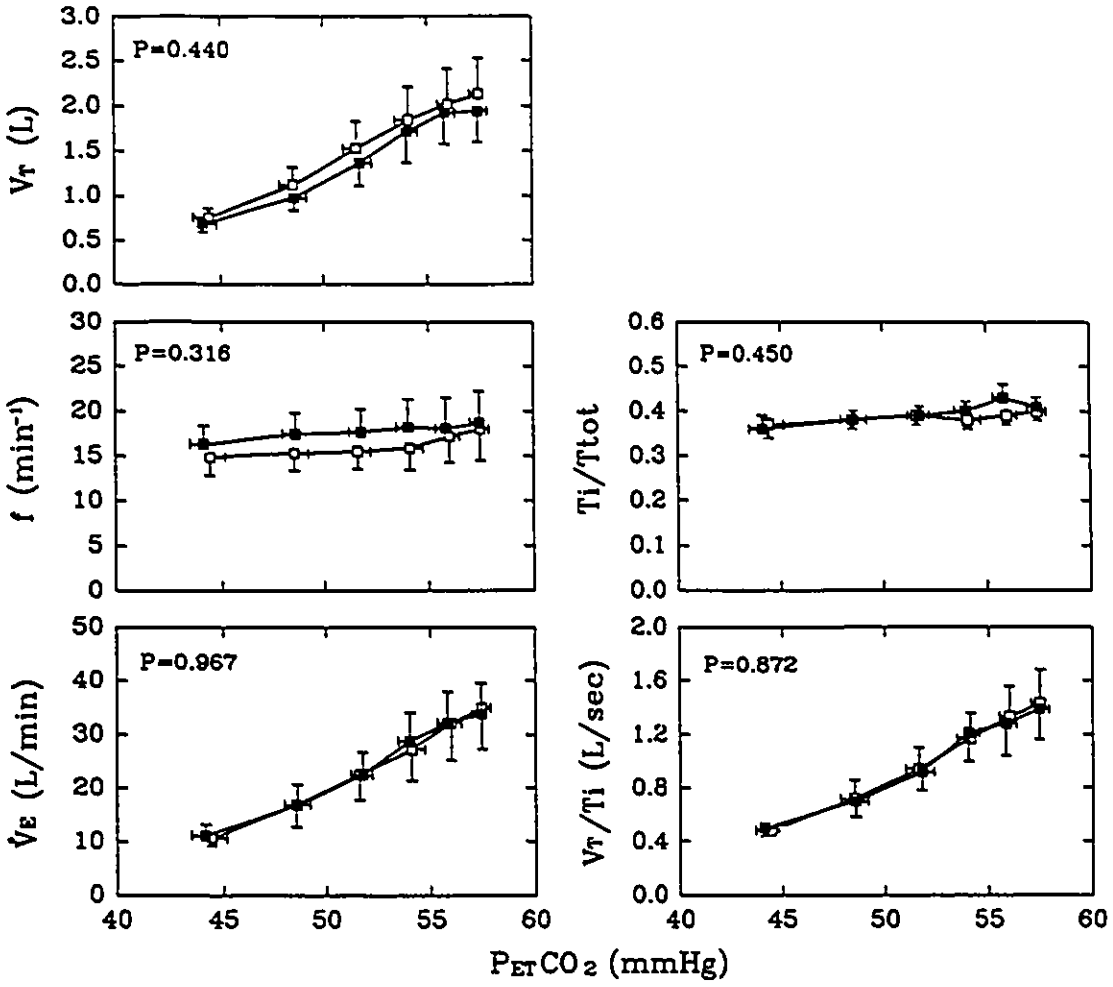

Figure 5.1: Minute ventilation, tidal volume, frequency, duty cycle and mean inspiratory flow as a function of $P_{ET}CO_2$ before and after diaphragm fatigue. The data shown are mean±s.e. from six subjects. On each panel, p-value is obtained using ANOVA for the comparison between fresh (open squares) and fatigued diaphragm (closed squares). V_T: tidal volume; f: breathing frequency: $\dot{V}_E$: minute ventilation; Ti/Ttot: inspiratory over total respiratory time; V_T/Ti: mean inspiratory flow; $P_{ET}CO_2$: end tidal partial pressure of CO_2.

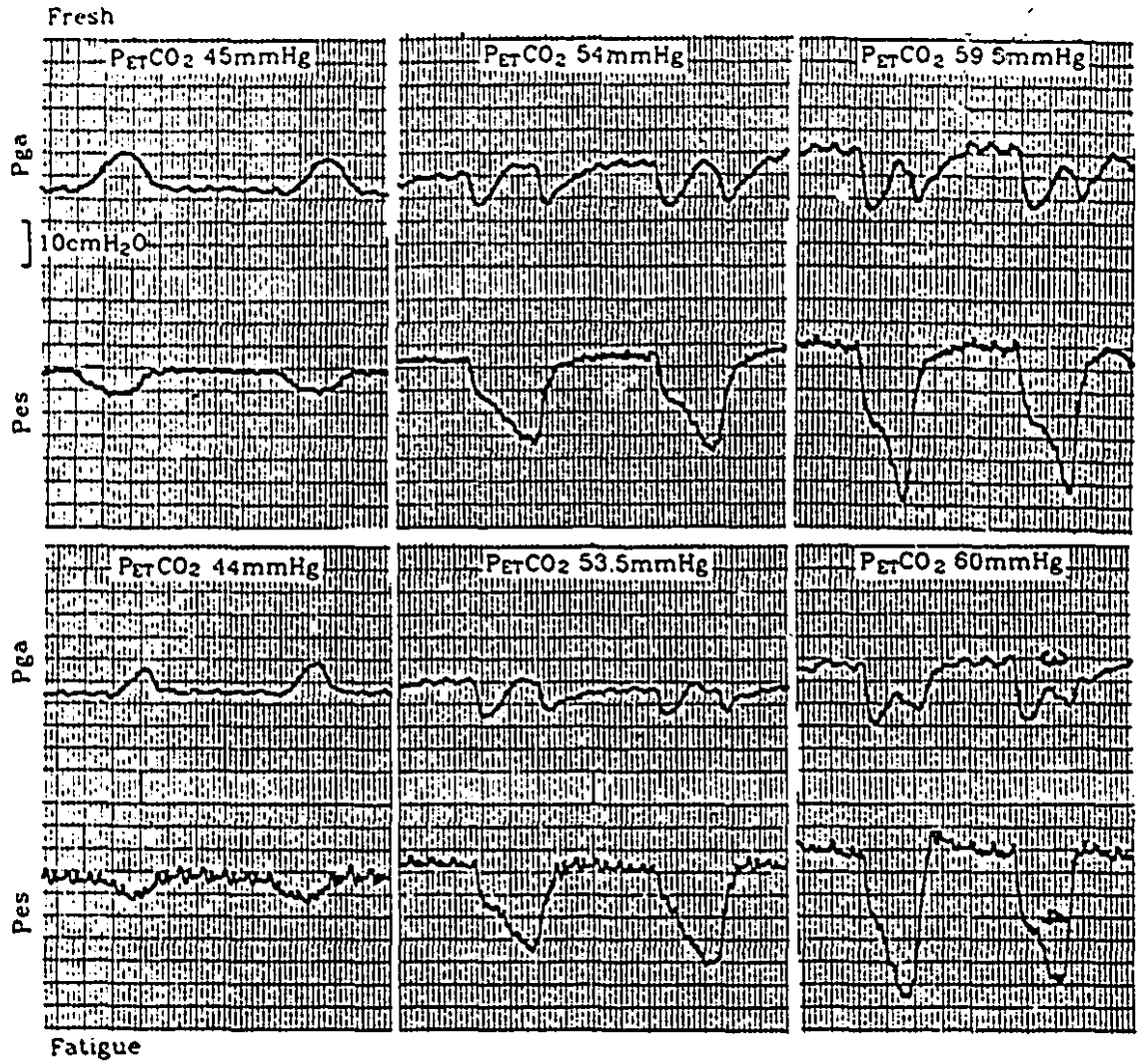

Figure 5.2: Experimental recordings of gastric pressure (Pga) and esophageal pressure (Pes) before (upper panel) and after diaphragmatic fatigue (lower panel) at 3 levels of CO_2 from one representative subject.

The only change in this pattern produced by diaphragmatic fatigue is a smaller inspiratory increase in Pga for the same inspiratory decrease in Pes. As a result, at a given level of $P_{ET}CO_2$ after fatigue, inspiratory Pdi swing was less. To produce a similar V_T and ΔPes in face of a diminished diaphragmatic contribution requires inspiratory rib cage muscle recruitment to a greater extent after fatigue than before.

This is further shown in figure 5.3 in which average values of ΔPes, ΔPga and ΔPdi during CO_2 rebreathing are plotted against $P_{ET}CO_2$. Before fatigue, ΔPga initially increased with increasing $P_{ET}CO_2$ and plateaued at a $P_{ET}CO_2$ of 51-52mmHg. After fatigue, ΔPga plateaued at lower $P_{ET}CO_2$ and eventually decreased at higher $P_{ET}CO_2$ levels. Furthermore, at a given $P_{ET}CO_2$ after fatigue, ΔPga was significantly reduced ($P<0.0001$). ΔPdi was also significantly reduced ($P<0.0005$). The decreased ΔPdi was fully accounted for by the decrease in ΔPga, so that ΔPes progressively increased with increasing $P_{ET}CO_2$ and remained unaltered at any given $P_{ET}CO_2$ after fatigue. As a result, the ΔPes/ΔPga ratio was significantly increased after diaphragmatic fatigue ($P<0.0001$).

I also measured absolute values of Pes and Pga at the start and at the end of inspiration (points of no flow) during CO_2 rebreathing and assumed that P_L was -Pes. Figure 5.4 shows the P_L-Pga relationships at 5 different levels of $P_{ET}CO_2$ before and after diaphragmatic fatigue. Before fatigue at the beginning of inspiration, there were a progressive decrease in P_L and increase in Pga with increasing $P_{ET}CO_2$, which was not significantly changed

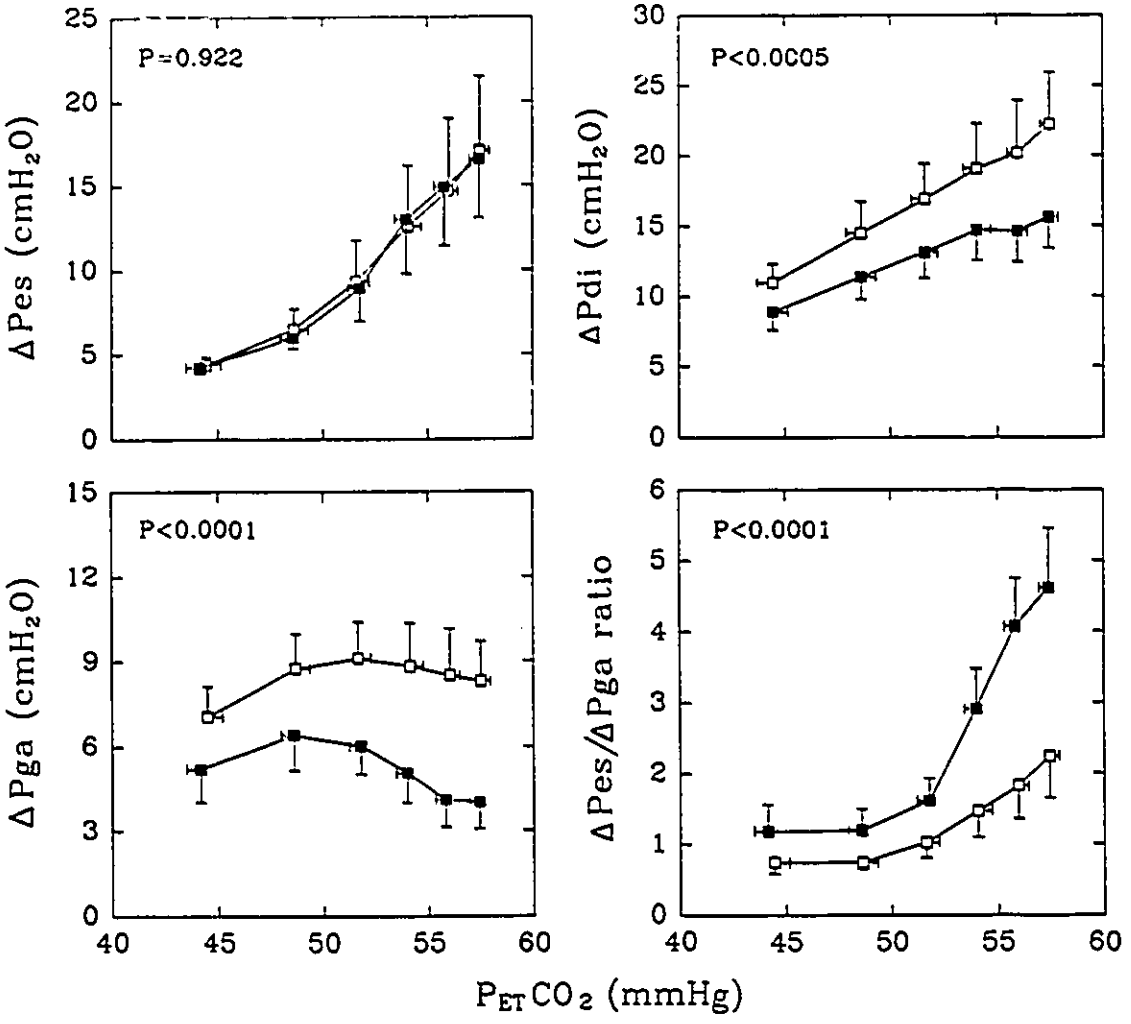

Figure 5.3: Change in gastric, esophageal and transdiaphragmatic pressure before and after diaphragm fatigue as a function of $P_{ET}CO_2$. The data shown are mean±s.e. from six subjects. ΔPes: tidal esophageal pressure change (expressed as magnitude independent of sign); ΔPga: tidal gastric pressure change; ΔPdi: tidal transdiaphragmatic pressure change. Other conventions are the same as in figure 5.1.

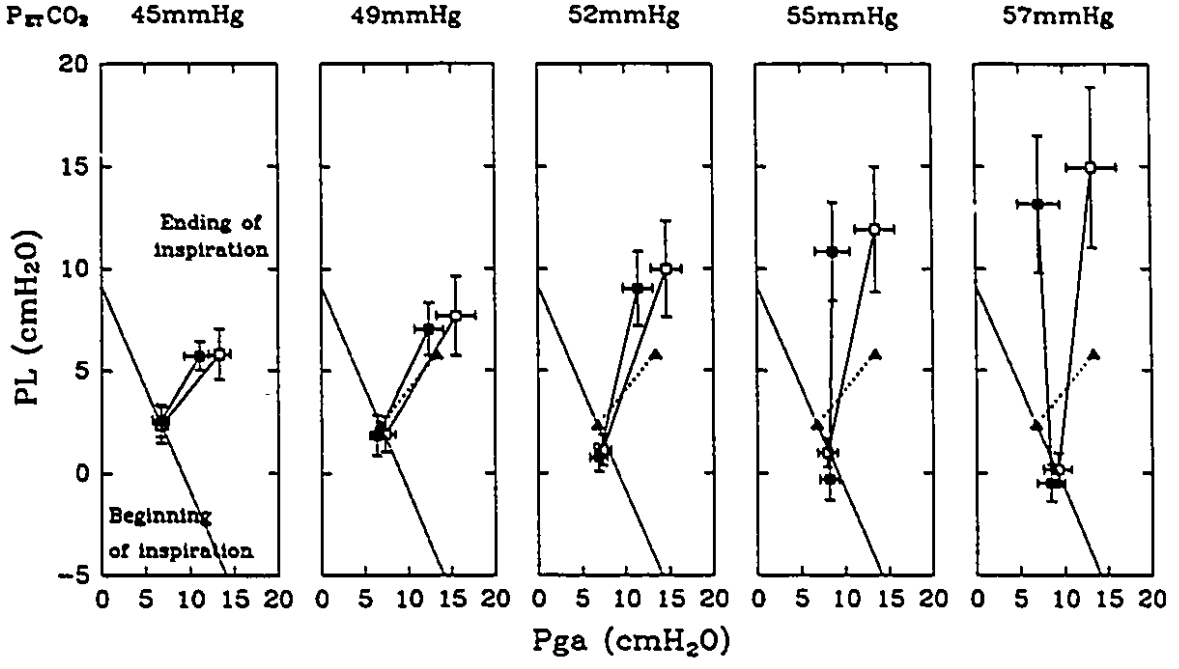

Figure 5.4: Relationship between transpulmonary pressure (P_L) and gastric pressure (Pga) before and after diaphragm fatigue at different values of $P_{ET}CO_2$. For the six subjects, the data shown are mean±s.e. of relationships determined at the start and at the end of inspiration (points of no flow) before (open squares) and after fatigue (closed squares) at five levels of end $P_{ET}CO_2$). The dotted line and triangles represent the PL-Pga relationship at the start of the CO_2 rebreathing before fatigue serving as control. Dashed line denotes iso-Pdi.

after fatigue. The starting point of P_L-Pga relationship at the same $P_{ET}CO_2$ before and after fatigue was not significantly different (Multivariate Test statistics with the Wilk's Lambda Test). I calculated the slope of this relationship from beginning to end inspiration. As shown in figure 5.4, there was a consistent increase in the slopes of P_L-Pga relationship after fatigue. At high $P_{ET}CO_2$, some slopes became negative (ΔPga was negative during inspiration) rendering the statistical analysis difficult. I therefore determined the angle between the P_L-Pga diagram and the X-axis and found that these angles were systemically greater after fatigue ($P<0.002$).

5.5 Discussion

The purpose of this investigation was to determine the influence of diaphragmatic fatigue on the chemical control of the respiratory muscles and the resulting ventilatory response to CO_2. The experimental intervention was the induction of diaphragmatic fatigue. The fall in Pdi,T after breathing against the fatiguing task as shown in table 5.1 indicates that I achieved the desired intervention. It has previously been demonstrated that recovery of diaphragmatic capacity to generate twitch (3,43) or low frequency force (2) after diaphragmatic fatigue is prolonged for more than one hour at FRC, which provides more than sufficient time to conduct the CO_2 rebreathing test. Thus I am confident that I successfully measured the ventilatory and respiratory muscle responses to CO_2 while the diaphragm was fresh and fatigued before any significant recovery. CO_2 itself has been shown to depress diaphragmatic contractility both in vitro (14) and in vivo (21). This occurred during more prolonged exposure to CO_2 than the rebreathing procedure we used which only took 3-4 min. In 2 subjects I measured Pdi,T amplitude before and after a CO_2 rebreathing procedure and found no change. Thus, it is unlikely that CO_2 rebreathing itself impaired diaphragmatic contractility in my study.

In spite of the successful induction of diaphragmatic fatigue my subjects showed no significant alteration in the ventilatory response to CO_2. As shown in figure 5.1, there was no significant change in V_T, f, $\dot{V}_E$, Ti/Ttot, and V_T/Ti so that the ventilatory

response to CO_2 was not altered by diaphragmatic fatigue.

On the other hand, there was a significant change in the muscles recruited to achieve the same $\dot{V}_E$ before and after fatigue. As shown by Macklem et al (27) the slope of the relationship P_L/P_{ga} can be used under quasi-static conditions to obtain a qualitative estimate of the relative contribution of the diaphragm, the rib cage inspiratory muscles, and the expiratory muscles to breathing.

If the expiratory muscles are relaxed during inspiration both before and after fatigue at any given level of CO_2, as suggested by figure 5.2, then the relationship between P_L and P_{ga} at zero flow points at beginning and end inspiration, can be used to analyze the relative contributions of the diaphragm and inspiratory muscles of the rib cage to breathing. Analysis of this diagram is based on the fact that both sets of muscles are synergistic in their effects on the lung, but antagonistic to the abdomen. When they contract, both act to inflate the lung as reflected in an increase in P_L, whereas contraction of the rib cage inspiratory muscles causes an inward abdominal displacement and a fall in P_{ga} while diaphragmatic contraction causes an outward displacement of the abdomen and an increase in P_{ga} (27,28). The slope P_L/P_{ga} therefore indicates the relative contributions of the rib cage inspiratory muscles vis-à-vis the diaphragm to inspiration.

During quiet breathing the rib cage muscles are recruited just enough so that the displacements of rib cage and abdomen are close to the relaxation line (11,26) as described by Konno and Mead (23). As a result $\Delta P_L/\Delta P_{ga}$ during quiet inspiration is greater than it is

during pure diaphragmatic contraction. With inspiratory rib cage muscle recruitment this ratio will increase, whereas with derecruitment it will decrease (27). With expiratory muscle recruitment during expiration bringing end-expiratory lung volume below FRC, end expiratory P_L decreases while Pga increases. As the diaphragm is relaxed at end expiration the P_L-Pga relationship in the P_L-Pga diagram will be displaced along the zero Pdi isopleth or somewhat to the right of it if there is significant passive tension in the diaphragm (7).

With this background I can now interpret the data in figures 5.2-5.4. As shown in figure 5.4, when ventilation was stimulated by CO_2 with the fresh diaphragm, $\Delta P_L/\Delta Pga$ slope progressively increased as $P_{ET}CO_2$ increased. Figure 5.3 shows that the diaphragm was recruited, but more of the pressure that it developed was converted into a fall in pleural pressure. In fact, beyond a $P_{ET}CO_2$ of 51-52 mmHg there was no further increase in Pga as Pdi increased. This can only be explained by a progressively and proportionately stronger recruitment of the inspiratory rib cage muscles than of the diaphragm as $P_{ET}CO_2$ increased.

In addition to the increase in slope of the $\Delta P_L/\Delta Pga$ relationship with increasing $P_{ET}CO_2$, there was a fall in end-expiratory P_L and an increase in Pga, which fell on or close to the zero Pdi isopleth (figure 5.4). This indicates that CO_2 recruits the expiratory muscles to lower end-expiratory lung volume in normal humans. As a result the early part of inspiration is passive and is performed by elastic energy stored within the chest wall and

requires no inspiratory muscle recruitment.

These findings are in keeping with the observations that the responses of the respiratory muscles to an increased external load (1,20,31) or an internal load by a posture change from supine to upright (42) are an augmentation of the activity of extra-diaphragmatic inspiratory muscles that is proportionately greater than of the diaphragm plus an increased activation of expiratory muscles (13,18,24,31). Similarly, with increasing drive to respiratory muscles such as during CO_2 rebreathing, the compartmental response of the rib cage is always relatively greater than that of the abdomen (8,9,33). Recruitment of the rib cage muscles not only assists the diaphragm because it takes over a part of the work of breathing, but also because it converts a greater proportion of the Pdi developed into a useful fall in pleural pressure. Since the inspiratory rise in abdominal pressure becomes less, the work done on the abdomen diminishes. In assisting the diaphragm, the inspiratory rib cage muscles may protect against diaphragmatic fatigue (42).

Similar compensatory mechanisms might also be present after diaphragmatic fatigue, a condition in which the load relative to diaphragmatic strength increases for a given level of ventilation. Since inspiratory muscles as a group are synergic when contracting against increased load to delay task failure (25,37), one may expect a substantial change of their recruitment following the fatigue of a single or a group of inspiratory muscles.

This is precisely what I found. After fatigue, ΔPdi and ΔPga

were diminished at all levels of ventilation; ΔPga reached a plateau at lower values of $P_{ET}CO_2$ and lower values of ΔPga; At higher values of $P_{ET}CO_2$, ΔPga actually decreased. However, ΔPes was unchanged. At all levels of ventilation, at a given value of $P_{ET}CO_2$ the slope $\Delta P_L/\Delta P_{ga}$ increased and at high levels of $P_{ET}CO_2$ the slope sometimes became negative. These changes were significant. However, the end-expiratory P_L and Pga at a given level of $P_{ET}CO_2$ was not affected by diaphragmatic fatigue.

I interpret these data as indicating an increased drive to the inspiratory rib cage muscles at a given level of $P_{ET}CO_2$ to compensate for the decrease in Pdi, in order to maintain V_T, V_T/T_i, and $\dot{V}_E$ constant. There was, however, no change in the drive to the expiratory muscles as a consequence of diaphragmatic fatigue.

I do not believe that the smaller Pdi after fatigue was due to shortening of diaphragmatic fiber length or an increase in the velocity of shortening. Greater shortening of the diaphragmatic fibers requires greater outward abdominal displacements during inspiration, not less (12,22). Although I did not measure abdominal displacements they were presumably less after diaphragmatic fatigue because ΔPga was less, and diaphragmatic fatigue does not change abdominal compliance (43). Therefore, diaphragmatic shortening during inspiration post-fatigue at any given level of $P_{ET}CO_2$ was probably less than before fatigue. As fatigue had no influence on breathing pattern, the velocity of shortening was also presumably less after fatigue since the degree of shortening decreased while inspiratory time remained constant. If the diaphragm was operating

at longer average length and with a smaller velocity of shortening, it would tend to maintain Pdi in spite of a decrease in diaphragmatic contractility. Clearly, this protective mechanism was not effective in maintaining the diaphragm's contribution to V_T and V_E.

Although I cannot be absolutely certain that I did not fatigue the inspiratory rib cage muscles during my experiments, I think that this is unlikely. The work of Fitting et al (15) is relevant to this concern. By monitoring the EMG high-to-low frequency ratio (H/L) of different inspiratory muscles, these authors demonstrated that the diaphragm and rib cage muscles could be separately fatigued dependent on the fatigue strategy used. In particular, inspiratory resistive loaded breathing with the diaphragmatic emphasis was inevitably associated with a fall in H/L of the diaphragm but a constant H/L of parasternal and sternocleidomastoid muscles (15). Furthermore, in chapter 6, I will show that global inspiratory muscle fatigue induced by generating a target mouth pressure with each breath produces a markedly different ventilatory response to CO_2 from that following diaphragmatic fatigue. In contrast to the present study I have found that global fatigue results in rapid shallow breathing and a marked decrease in end-expiratory lung volume during CO_2 rebreathing (chapter 6). Global fatigue has been previously demonstrated to cause rapid shallow breathing at rest (16,37) and during exercise (29,30). However, little data are available for the effect of diaphragmatic fatigue on breathing pattern except while performing the fatiguing task

under conditions when the ventilatory pattern was not imposed on the subject (38).

Although I am confident that diaphragmatic fatigue caused an increased drive to the inspiratory rib cage muscles, I cannot state whether diaphragmatic drive remained constant, increased or diminished. I tried to determine the EMG activity of the diaphragm in this study but I found it difficult to draw conclusions from the data for several reasons. First, it has been shown that the EMG signal of the diaphragm (compound mass action potentials) measured by esophageal electrodes changes substantially with alterations in lung volume (17) and the degree of voluntary effort (5); both alterations are characteristic during CO_2 rebreathing. Secondly, diaphragmatic EMG obtained from an esophageal electrode is contaminated by substantial cardiac artifacts, which can be eliminated to a large extent by a low frequency cutoff up to 60-100Hz. This could not be done in the present study because diaphragmatic fatigue is associated with a progressive shift of its EMG power spectrum to lower frequencies (19). Thus the signal to noise ratio was unacceptably high. Thirdly, even slight changes in esophageal electrode position or orientation might profoundly affect the recorded signal (17). I cannot be certain that such changes did not occur after our fatigue protocol involving massive diaphragmatic inspiratory efforts and abdominal muscle activation. In the presence of rib cage and abdominal muscle activity surface electrodes cannot be used to estimate diaphragmatic drive either. For these reasons I gave up my efforts to measure diaphragmatic

EMG, so that I do not know whether diaphragmatic activation changed or not. Inasmuch as this is concerned, it should be noted that central factors have been found to play a role in the development of diaphragmatic fatigue in both animal (36) and human (6) in vivo studies. This mechanism may serve to protect the already fatigued respiratory muscles from further deterioration at the cost of CO_2 retention. This appears to be the case in some COPD patients (4). In addition, hemidiaphragmatic fatigue induced by hemidiaphragmatic ischemia in anaesthetized dogs (39) resulted in a reduction in EMG activity from the affected hemidiaphragm while that from the fresh contralateral hemidiaphragm and from parasternal intercostal muscles increased. Reperfusion of the phrenic artery resulted in a return of each of the above mentioned EMG's towards baseline levels (39).

In summary, I found that the major compensatory mechanism for diaphragmatic fatigue during CO_2 rebreathing is the proportionately greater use of inspiratory rib cage muscles. By doing so, $\dot{V}E$, f, V_T, V_T/Ti, and Ti/Ttot remained constant. As has been demonstrated previously (18,24,40,41), there is a progressively increased recruitment of expiratory muscles with increasing CO_2 stimulus, but this pattern of expiratory muscle activity was not modified by diaphragmatic fatigue.

It needs to be pointed out that these results only pertain to normal subjects under the present experimental conditions. They should not be extended to patients with impaired respiratory function and to conditions with higher ventilatory requirements or

with greater impairment of diaphragmatic contractility.

Nevertheless, it is interesting that there is a strong tendency to preserve ventilation and the parameters that determine it. It is clear that the control system is plastic and can adapt to different adverse conditions to maintain minute ventilation, frequency, tidal volume, duty cycle, and mean inspiratory flow. What receptors are involved in "measuring" ventilation so that it can be preserved and what switching mechanisms are present in the central nervous system to alter the drive to the respiratory muscles in an appropriate way are important questions that deserve further research.

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CHAPTER 6

EFFECT OF GLOBAL INSPIRATORY MUSCLE FATIGUE ON RESPIRATORY MUSCLE CONTROL AND VENTILATORY RESPONSE DURING CO_2 REBREATHING

In chapter 5 I examined the effect of diaphragmatic fatigue on respiratory muscle recruitment and ventilatory response during CO_2 rebreathing. I showed that the only change after diaphragmatic fatigue was greater recruitment of inspiratory rib cage muscles, which fully compensated for the decreased diaphragmatic contractility due to fatigue, so that the ventilation and its pattern were preserved. Since pure diaphragmatic fatigue probably only occurs in laboratories, in chapter 6 I will present the results demonstrating the alterations of ventilation and its control after global inspiratory muscle fatigue, a condition in which all or the great majority of inspiratory muscles (the diaphragm, the inspiratory rib cage muscles, and probably accessory muscles) are fatigued.

6.1 Abstract

I evaluated the effect of global inspiratory muscle fatigue on ventilation and respiratory muscle control during CO_2 rebreathing in six seated normal subjects. Fatigue was induced by developing 80% maximal inspiratory pressure ($P_{I,max}$) against an inspiratory resistance with each breath until exhaustion. Fatigue was demonstrated by a reduction in transdiaphragmatic pressure (Pdi) in response to bilateral supramaximal phrenic nerve shocks and in $P_{I,max}$. CO_2 response curves were measured before and after fatigue. Diaphragm recruitment was assessed by ΔPdi, inspiratory rib cage muscle recruitment by the changes in transpulmonary pressure (ΔP_L) relative to ΔPdi and ΔPga; end-expiratory muscle recruitment by end-expiratory ΔP_L. Global inspiratory muscle fatigue caused a decrease in tidal volume (V_T) and increased breathing frequency in response to CO_2 but did not change minute ventilation ($\dot{V}_E$), duty cycle, and mean inspiratory flow. The decreased V_T resulted from derecruitment of both rib cage muscles and diaphragm. End-expiratory P_L decreased more after fatigue indicating an additional decrease in end-expiratory lung volume due to expiratory muscle recruitment. Thus, the initial portion of inspiration was passive. This combined with the reduction in V_T decreased the fraction of V_T attributable to inspiratory muscle recruitment to ~50% of control, the elastic work performed by the inspiratory muscles per breath to ~30% of control, and inspiratory muscle elastic power to ~40% of control. I conclude that respiratory control mechanisms are plastic and that the respiratory centres alter their control in a manner

appropriate to the contractile state of the respiratory muscles in order to conserve the V_E response to CO_2.

KEY WORDS Diaphragm, rib cage muscles, expiratory muscles, recruitment, end-expiratory lung volume.

6.2 Introduction

I have demonstrated in normal subjects that diaphragmatic fatigue alone does not change the ventilatory response to CO_2 but leads to inspiratory rib cage muscle recruitment while the diaphragmatic contribution is diminished (chapter 5). This suggests that the brain stem respiratory centres can adjust their output to the inspiratory muscles in a manner appropriate to the contractile state of the diaphragm vis-à-vis the inspiratory muscles of the rib cage.

Since respiratory muscles are synergic in breathing against a load (35), pure diaphragmatic fatigue should not be present in most patients who may develop inspiratory muscle fatigue. Instead, most of the inspiratory rib cage muscles in addition to the diaphragm should eventually become fatigued, a condition referred to in the present study as global inspiratory muscle fatigue. Global inspiratory muscle fatigue has been studied in normal subjects (21,35) and suspected to occur in patients having weaning difficulties (14); rapid shallow breathing was reported in both groups. The combination of fatigue and rapid shallow breathing which diminishes alveolar ventilation for any value of minute ventilation (V_E) may be important in the development of hypercapnic respiratory failure (35,36). It is therefore important to understand the effect of global fatigue on control of ventilation.

Based upon my study of the effect of diaphragmatic fatigue on ventilatory control, I hypothesized that global fatigue would result in a substantial alteration of respiratory centre output and

respiratory muscle control. I performed this study to test this hypothesis. I demonstrate that the responses to CO_2 after global fatigue are different in a number of ways from those after diaphragmatic fatigue (chapter 5).

6.3 Methods

Subjects:

Six normal male subjects (mean age: 35.2 yr), having given their informed consent, participated in the present study. Three of them did not know exactly the purpose of the study. The study was approved by the Ethics Committee of Montreal Chest Hospital.

Experimental setup:

Measurements: The subjects were comfortably seated in an armchair and breathed with a noseclip from a mouthpiece connected to a two way valve system. One port of the two way valve was connected to the rebreathing bag and the other port to room air. Esophageal (Pes) and gastric pressures (Pga) were measured by means of conventional balloon-catheters (32) attached separately to two differential pressure transducers (Validyne). Transdiaphragmatic pressure (Pdi) was obtained by electrical subtraction of Pes from Pga. A third pressure transducer was connected to the mouthpiece to measure mouth pressure (Pm). All pressures were measured relative to atmospheric pressure. Flow was measured by a pneumotachograph (Fleisch #2) attached between the mouthpiece and the two way valve system. Lung volume change was obtained by integrating the flow. For 5 subjects, the changes in anterior-to-posterior (AP) dimensions of the rib cage and abdomen were monitored by magnetometers. The pair of magnetometer coils that recorded the displacement of the rib cage was fixed at midline at the nipple level and the other pair at the midline 1-2 cmH_2O above the umbilicus. These magnetometer signals were calibrated using iso-

volume manoeuvres as described by Konno and Mead (27). End-tidal CO_2 concentration was continuously monitored at the mouthpiece with a carbon dioxide analyzer system (Ametek CD-3A) which was calibrated before and sometimes during the test with standard and chemically analyzed gas mixtures. End-tidal CO_2 partial pressure ($P_{ET}CO_2$) was calculated assuming that the atmospheric pressure was 760mmHg and that the expired gas was fully saturated with water vapour.

CO_2 rebreathing: CO_2 rebreathing was performed using Read's method (34). Briefly, the subjects rebreathed from a bag which contained 6-8 litres of gas made up of 7% CO_2 and 93% O_2. The procedure generally lasted for 3-4 minutes and was terminated at an end-tidal CO_2 concentration around 8% corresponding to a $P_{ET}CO_2$ about 57mmHg. No major discomfort was reported by our subjects in association with the procedure.

Induction of fatigue: Global inspiratory muscle fatigue was induced by breathing through a large inspiratory resistive load. Expiration was unloaded. During each loaded inspiration the subjects were instructed to develop and maintain throughout the inspiration a square-wave Pm that was 80% of the subjects' maximal inspiratory pressure ($P_{I,max}$) with a duty cycle of 0.6. This target breathing pattern was illustrated to the subjects on a storage oscilloscope. The fatigue task was terminated when the subjects were unable to reach and maintain the target for more than 3 consecutive breaths and/or the subjects felt that they could not continue and came off the mouthpiece in spite of verbal encouragement by the operators.

Assessment of inspiratory muscle strength: Inspiratory muscle strength before and after the fatigue task was assessed by measuring $P_{I,max}$ (8) and Pdi twitch amplitude (Pdi,T) in response to supramaximal bilateral phrenic nerve shocks at functional residual capacity (FRC) against an occluded airway. The detailed description of the phrenic nerve stimulation technique for Pdi,T measurement can be found in the literature (6,7).

Experimental protocol:

At the beginning of each test, the subject breathed room air through the mouthpiece with the noseclip for a few minutes until all the measured variables stabilized. Then the two way valve from which the subject was breathing was switched to the rebreathing bag with the subject uninformed and the CO_2 rebreathing test began. This test generally took 3-4 minutes and was terminated when end-tidal CO_2 concentration was around 8%. The subjects were given at least 10 minutes rest after the first CO_2 rebreathing run and then performed the global inspiratory muscle fatigue task as described. After the fatigue task, the same CO_2 rebreathing procedure was repeated. In all cases, the second rebreathing test was completed within 15 minutes after the end of the fatiguing task, that is before there was any significant recovery from low frequency fatigue (40). Before and after the fatigue task prior to the second CO_2 rebreathing, $P_{I,max}$ was determined in all subjects and Pdi,T in five subjects.

Data recording and analysis:

Pressures, flow, magnetometer signals, and end-tidal CO_2 were

recorded on an eight-channel strip chart recorder (Hewlett Packard) and on a magnetic tape recorder (Hewlett Packard). These signals were passed through a 12 bit A-D converter and digitized and analyzed at 200Hz by a computer. Pdi and tidal volume (V_T)were generated on computer by subtracting Pes from Pga and by integrating the flow, respectively. The volume was corrected to B.T.P.S. conditions. $P_{I,max}$ was read directly from the paper recordings. Pdi,T was sampled at 500Hz by computer and averaged and measured as the peak amplitude relative to its immediate preceding baseline. The data during CO_2 rebreathing were analyzed breath-by-breath.

The ventilatory parameters included V_T, breathing frequency (f), $\dot{V}_E$, duty cycle (Ti/Ttot, where Ti: inspiratory time and Ttot: total respiratory cycle time), and mean inspiratory flow (V_T/Ti).

Respiratory muscle response to CO_2 was assessed by pressure changes and rib cage and abdominal displacements. Tidal change of Pes, Pga, and Pdi (ΔPes, ΔPga, and ΔPdi) were measured at zero flow points at beginning and end expiration. Diaphragmatic response was assessed by ΔPdi changes. The response of the inspiratory rib cage muscles was assessed by changes in P_L or Pes relative to Pdi and Pga during inspiration and by chest wall displacements. Expiratory muscle response to CO_2 was estimated by end-expiratory P_L and Pga. The data are expressed as mean±s.e.

6.4 Results

$P_{I,max}$ and Pdi,T measured before and after global fatigue task are shown in table 6.1. Both pressures were significantly reduced after the fatigue task.

Table 6.1: Inspiratory muscle strength.

Subjects	$P_{I,max}$ (cmH_2O)		Pdi,T (cmH_2)	
	Fresh	Fatigue	Fresh	Fatigue
1	122	83	17.8	10.7
2	125	90	30.2	23.5
3	124	98	17.9	10.9
4	110	82	26.6	17.1
5	113	84	28.5	17.6
6	105	84		
Mean	117	87	24.2	16.0
S.E.	3	3	2.6	2.4

$P_{I,max}$: maximal inspiratory pressure; Pdi,T: transdiaphragmatic pressure twitch amplitude in response to bilateral supramaximal phrenic nerve single shocks. The measurements were made at end-expiratory lung volume with closed airways and expressed as cmH_2O. $P_{I,max}$ was chosen from the maximal record from at least three efforts. Pdi,T was the average of at least five twitches.

The ventilatory parameter response to CO_2 including V_T, f, $\dot{V}_E$, Ti/Ttot, and V_T/Ti are shown in figure 6.1. All subjects increased f and five subjects decreased V_T in response to CO_2 after the fatigue task. The remaining subject had an unchanged V_T response after fatigue. This was repeated twice. As a group, global fatigue produced a decrease in V_T and an increase in f ($P < 0.05$) and constant $\dot{V}_E$, Ti/Ttot, and V_T/Ti for a given $P_{ET}CO_2$.

Figure 6.2 shows the results of ΔPes, ΔPga, and ΔPdi (expressed as an amplitude independent of sign) measured at zero flow points at beginning and end-inspiration during CO_2 rebreathing. Before fatigue, ΔPes increased linearly with increasing $P_{ET}CO_2$ while ΔPdi initially increased but levelled off at high CO_2 drive, indicating preferential recruitment of inspiratory rib cage muscles with increasing $P_{ET}CO_2$. These findings are in general agreement with the CO_2 response reported in chapter 5, although differing in some details. After fatigue, ΔPes was significantly reduced and tended to plateau at high CO_2 drive. ΔPdi was also reduced; it reached a peak at $P_{ET}CO_2$ of 53mmHg and decreased thereafter. The reduction of both ΔPes and ΔPdi at a given $P_{ET}CO_2$ was significant ($P < 0.05$) indicating derecruitment of both the inspiratory rib cage muscles and the diaphragm. ΔPga was also significantly decreased after fatigue. The interpretation of this finding is not so clear, because as shown below Pga at the beginning of inspiration was higher at any given $P_{ET}CO_2$ after global fatigue than before.

Figure 6.3 shows the rib cage and abdomen compartmental V_T

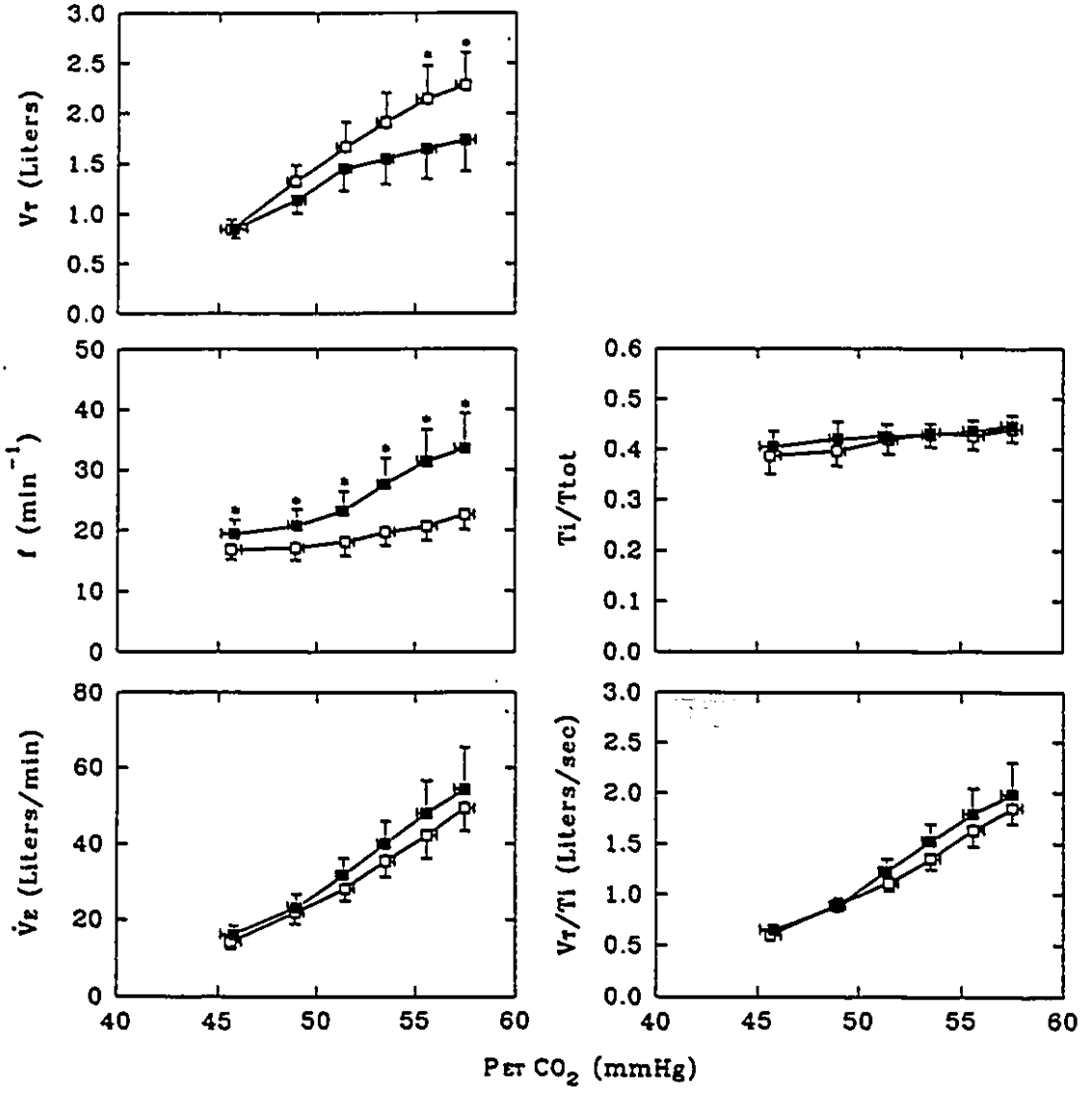

Figure 6.1: Ventilatory parameter response to CO_2 plotted as a function of partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) before (open squares) and after global inspiratory muscle fatigue (closed squares). V_T: tidal volume; f: breathing frequency; $\dot{V}_E$: minute ventilation; Ti/Ttot: duty cycle (the ratio of inspiratory time to total respiration cycle time); V_T/Ti: mean inspiratory flow. * donates $P < 0.05$.

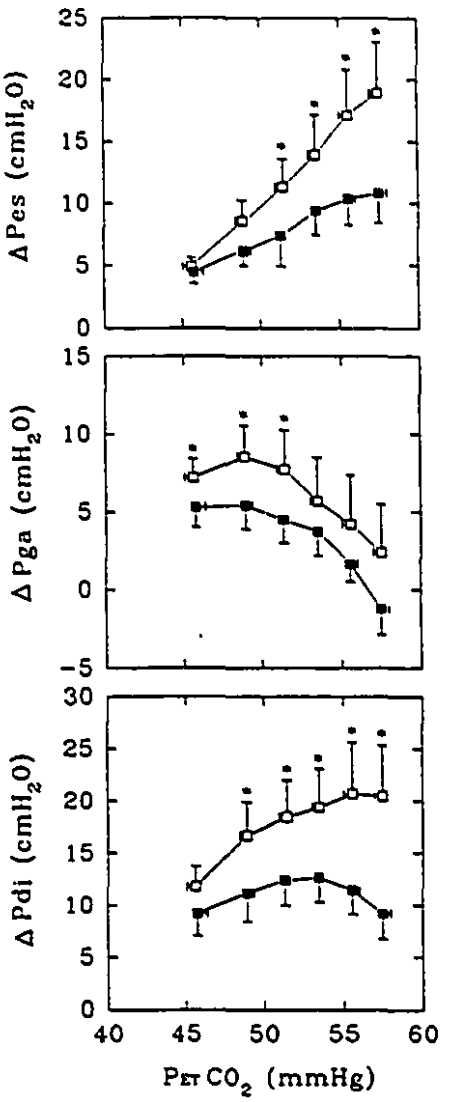

Figure 6.2: Inspiratory pressure changes determined between zero flow points. Pes: esophageal pressure; Pga: gastric pressure; Pdi: transdiaphragmatic pressure. The data are shown independent of sign. Other conventions are the same as in figure 6.1.

response to CO_2. One of the five subjects studied whose V_T response kept constant after fatigue did not show any change in chest wall displacement. The decreased V_T response to CO_2 after fatigue in the remaining four subjects was fully accounted for by a significant decrease in the rib cage V_T response with the abdominal V_T unaltered. Consequently, as a group, these subjects had a significant decrease in rib cage V_T at high CO_2 drive after global fatigue.

Figure 6.4 shows experimental recordings during CO_2 rebreathing from one subject. At high CO_2 drive ($P_{ET}CO_2$ 55mmHg in this figure), an inspiratory dip of Pga with outward displacement of abdomen was seen both before and after fatigue. This fall in Pga was accompanied by a rapid decrease in Pes, followed by a further slower decline. A similar pattern was observed in chapter 5 as shown in figure 5.2. At this point peak inspiratory flow had already been achieved with a substantial fraction of V_T already inspired. After fatigue the fall in Pga and Pes in this interval was greater and more prolonged while the fraction of V_T inspired was greater. This occurred at the beginning of inspiratory flow and therefore reflects expiratory muscle relaxation. The V_T generated in this interval was a result of energy stored in the respiratory system, by breathing below the equilibrium volume. As a result, the stored elastic energy was available to perform some of the work of inspiration. This component of V_T was greater after global fatigue.

Figure 6.5 shows the P_L-Pga diagram measured at end

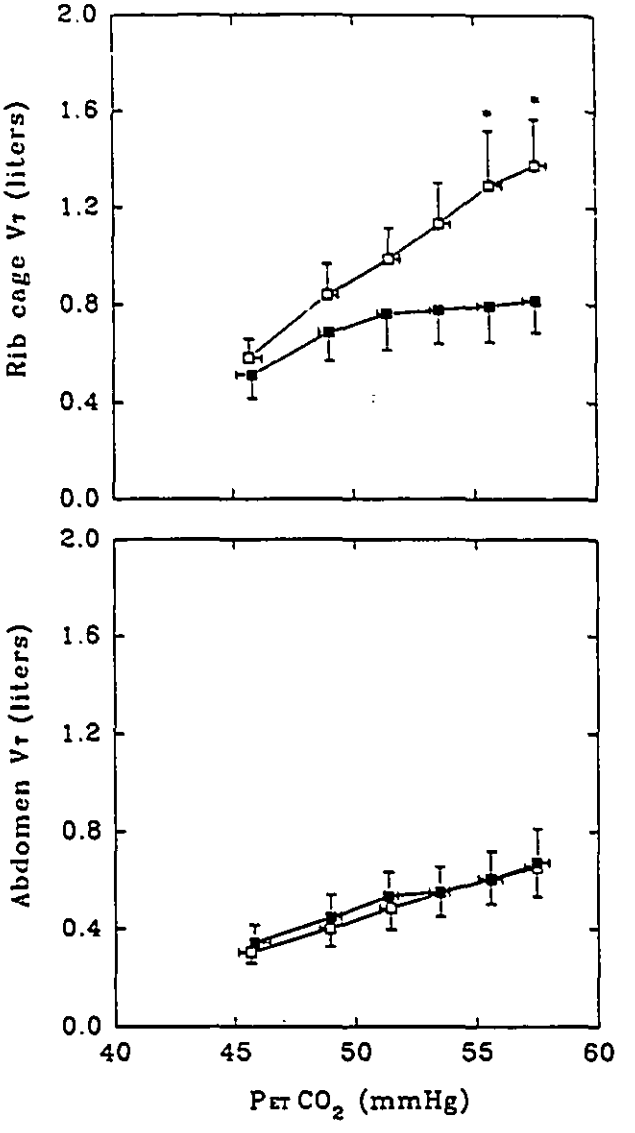

Figure 6.3: Rib cage and abdominal contributions to V_T during CO_2 rebreathing measured using magnetometers and calibrated with iso-volume manoeuvres. Other conventions are the same as in figure 6.1.

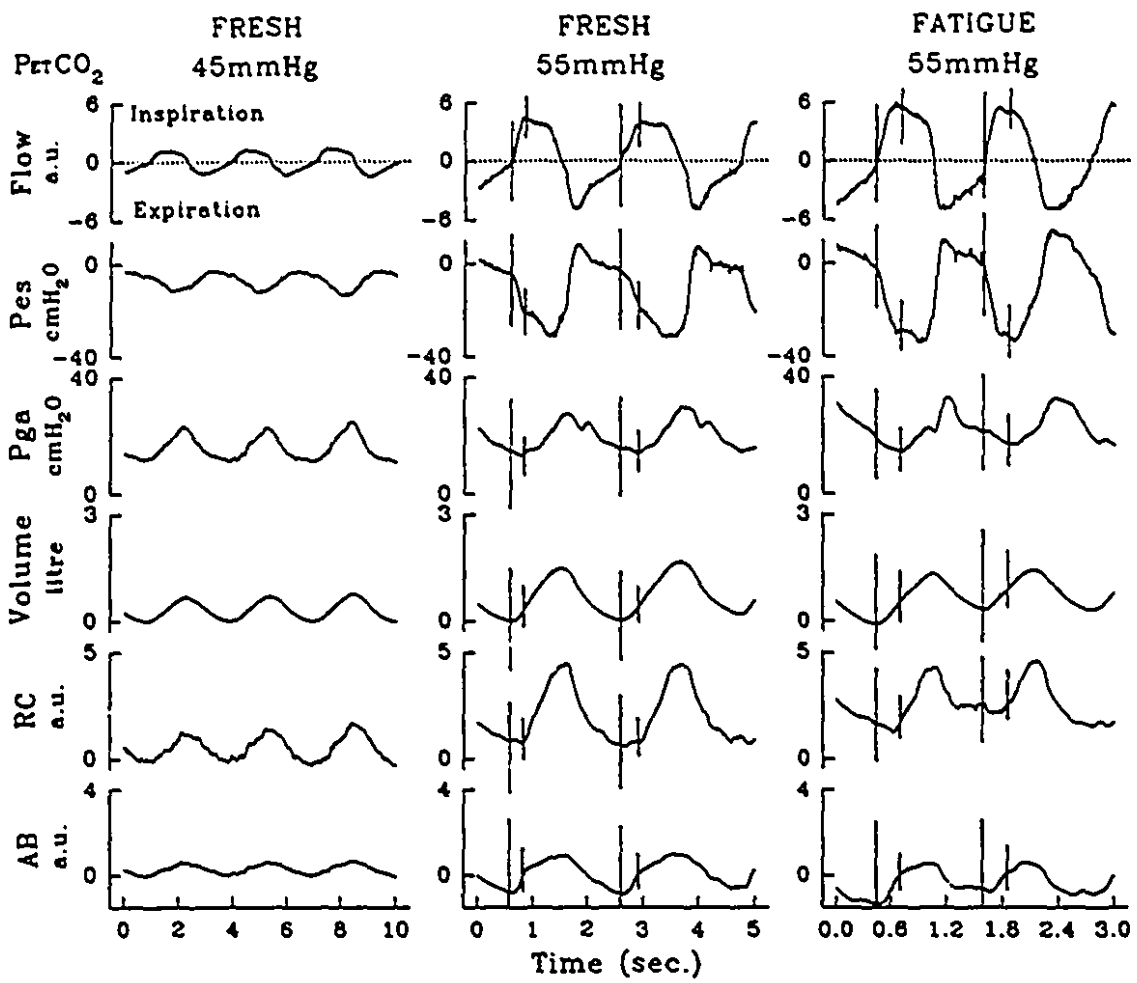

Figure 6.4: Experimental recording during CO_2 rebreathing from one subject. Pes: esophageal pressure; Pga: gastric pressure; RC: rib cage displacement; AB: abdominal displacement; $P_{ET}CO_2$: end-tidal partial pressure of CO_2. At the beginning of inspiratory flow an inspiratory dip of Pga is seen at $P_{ET}CO_2$ 55mmHg and occurs with outward displacement of the abdomen. The long vertical bars indicate the beginning of inspiratory flow and the short ones the end of the inspiratory dip of Pga. The increase in lung volume in this interval represents the V_T generated by expiratory muscle recruitment and is greater after (right panel) than before global inspiratory muscle fatigue (middle panel).

expiration. With increasing $P_{ET}CO_2$ there was a progressive decrease in P_L and an increase in Pga at end-expiration. After global fatigue, P_L was smaller and Pga greater resulting in a shift of the P_L-Pga relationship downwards and to the right, indicating that global fatigue and increasing CO_2 result in a greater recruitment of expiratory muscles and a greater decrease in end-expiratory lung volume than an increase in $P_{ET}CO_2$ alone. This is consistent with the greater inspiratory dip of Pga at beginning of inspiration after global fatigue (figure 6.4).

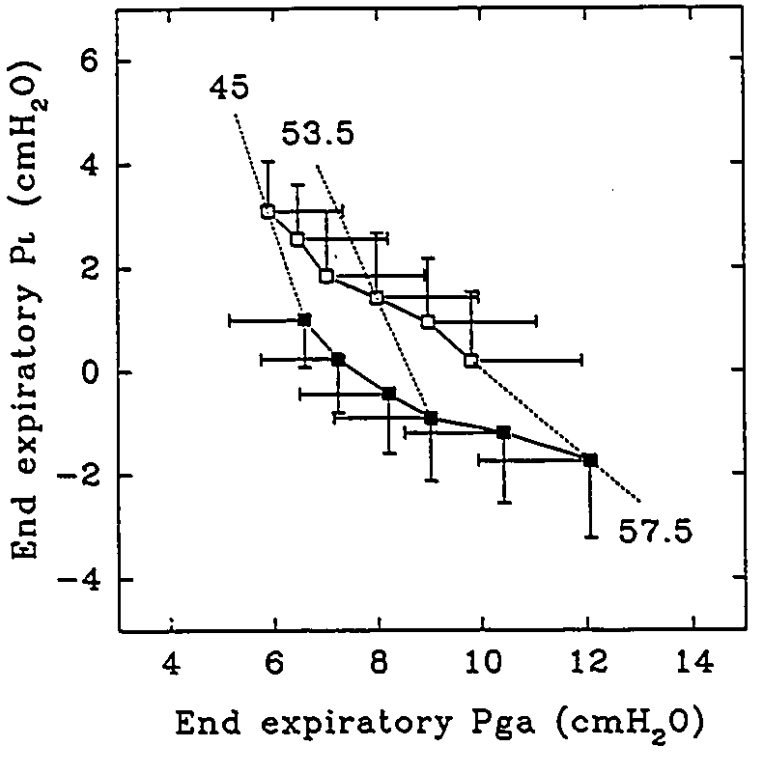

Figure 6.5: End-expiratory transpulmonary pressure (PL)-gastric pressure (Pga) relationships during CO_2 rebreathing. Dotted lines with numbers are the lines of iso-$P_{ET}CO_2$ (mmHg). Other conventions are the same as in figure 6.1.

6.5 Discussion

In chapter 5, I have shown that diaphragmatic fatigue did not change breathing pattern and expiratory muscle recruitment during CO_2 rebreathing in spite of significant decrease in diaphragmatic contractility; however, the unchanged pattern of ventilation was accomplished by recruiting inspiratory rib cage muscles indicating plasticity of respiratory centre output in response to changes in respiratory muscle contractility. In the present study, I have demonstrated that after global inspiratory muscle fatigue, rapid shallow breathing developed and expiratory muscles were recruited while both the diaphragm and the inspiratory rib cage muscles decreased their contribution to breathing during CO_2 rebreathing. Although these changes are quite different from those after diaphragmatic fatigue (chapter 5), minute ventilation remained constant indicating that this parameter is conserved despite marked changes in respiratory muscle contractility.

In this study, I fatigued the subjects' inspiratory muscles by inspiratory resistive loaded breathing with mouth pressure as a target. Several reasons lead me to believe that the great majority of the inspiratory muscles including the diaphragm, the inspiratory rib cage muscles, and probably the accessary muscles were fatigued at the limit of endurance. Firstly, I employed a standard fatigue protocol originally used by Roussos et al (35) and generally accepted and used by most investigators to induce global inspiratory muscle fatigue. It has been shown that during such a protocol, the diaphragm and the inspiratory rib cage muscles were

alternately recruited to generate the targeted mouth pressure (35). This strategy which was encouraged throughout the fatigue task of the present study presumably leads eventually to fatigue of both the diaphragm and rib cage muscles. Secondly, I showed that following the fatigue task, both Pdi,T and $P_{I,max}$ were significantly decreased indicating both diaphragmatic fatigue (5,40) and rib cage muscle fatigue. Since at present it is not known whether or not diaphragmatic fatigue alone affects $P_{I,max}$ and there is, as yet, no available method to separately determine rib cage muscle contractility, a reduced $P_{I,max}$ is generally thought to reflect a global decrease in inspiratory muscle strength involving both diaphragm and other inspiratory muscles (8,10,35). Thirdly, I found that the response to CO_2 following the present fatigue protocol was different in several important respects from those after diaphragmatic fatigue (chapter 5). Thus the effects induced by fatigue in the present protocol were qualitatively different from those induced by diaphragmatic fatigue. It could be argued, though, that rapid shallow breathing and expiratory muscle recruitment which were not seen after diaphragmatic fatigue (chapter 5) could be due to a greater degree of diaphragmatic fatigue in the present study. However, Pdi,T decreased by 35% after the global fatigue protocol which was comparable with the decrease of 32% after diaphragmatic fatigue (chapter 5), indicating that the diaphragm was fatigued to a similar extent. Then the "greater" or additional fatigue in the present study was due to fatigue of inspiratory rib cage muscles.

Following diaphragmatic fatigue (chapter 5), I applied the P_L-Pga relationship determined at zero flow points to infer changes in recruitment of respiratory muscles during CO_2 rebreathing (30). I demonstrated an increased slope of the P_L-Pga relationship accompanied by a decrease in Pdi after diaphragmatic fatigue indicating a proportionately greater recruitment of rib cage muscles to unload the fatigued diaphragm and to maintain the V_T, f, and $\dot{V}_E$ responses to CO_2 constant. Expiratory muscle recruitment was the same before and after diaphragmatic fatigue.

In the present study, the end-expiratory P_L-Pga relationship was shifted downwards and to the right for a given $P_{ET}CO_2$ after global inspiratory muscle fatigue (figure 6.5), indicating additional expiratory muscle recruitment. As a result, changes in the magnitude of inspiratory muscle recruitment cannot be confidently estimated by comparing slopes of P_L-Pga relationships (30). However, I found that both tidal ΔPdi and ΔPes significantly decreased for a given $P_{ET}CO_2$ after global fatigue (figure 6.2). Because diaphragmatic fatigue alone did not affect the tidal ΔPes response to CO_2 (chapter 5), these results suggest that after global fatigue, both the diaphragm and the inspiratory rib cage muscles generated less force for a given CO_2 stimulus resulting in the decreased V_T response to CO_2.

The derecruitment of rib cage muscles of my subjects is also demonstrated by the fact that the decreased V_T response to CO_2 was fully accounted for by a decrease in rib cage but not in abdominal compartmental response to CO_2 after global fatigue (figure 6.3).

The importance of the rib cage muscles for augmented ventilation has been shown in several studies. In animals of different species (15-18,39), increasing CO_2 progressively increases activation of rib cage muscles over that of the diaphragm. Studies in humans (11,12,33) have demonstrated that V_T response to CO_2 is largely determined by rib cage compartmental response and the rib cage response slope is always greater than that of abdomen. Similar results were also seen during hypoxia (11,13) and exercise (11). Hussain and Pardy (25) have demonstrated that inhibition of rib cage muscle action by rib cage strapping markedly reduced exercise endurance. In addition, with added loads, inspiratory muscles of the rib cage are preferentially recruited as assessed both by electromyography (EMG) (4,24) and by chest wall compartmental response (31). These studies suggest that inspiratory rib cage muscles play an increasingly important role with increasing ventilation or during load compensation. My results prior to fatigue showing a plateau in the Pdi response to CO_2 while ΔPes and V_T continued to increase (figure 6.2), are consistent with these studies. Furthermore, when only the diaphragm was fatigued, additional rib cage muscle recruitment fully preserved the ventilatory response to CO_2 and no other adjustments of the ventilatory control system were made (chapter 5).

In contrast, with global fatigue, the control system abandoned its reliance on the rib cage muscles, so that additional mechanisms, namely decreasing V_T, increasing f, and recruitment of expiratory muscles were required to keep $\dot{V}_E$ response to $\dot{C}O_2$

constant. Indeed, it is remarkable that this relationship is so well conserved in the face of marked variation in respiratory muscle contractility.

The strategy adopted by the control system in global fatigue is to decrease the requirement of inspiratory muscle recruitment to achieve an increase in $\dot{V}_E$. This was accomplished both by decreasing V_T and end-expiratory lung volume. To maintain $\dot{V}_E$ constant under these circumstances requires an increase in f, resulting in rapid shallow breathing. This has been reported in normal human subjects after a similar fatigue protocol to mine (21), in patients during weaning (14,38), and in animals breathing against high inspiratory resistive loads (2,37). With such a breathing pattern, a smaller V_T requires a smaller intrathoracic pressure change as I showed in the present study. The fatigued inspiratory muscles are thus unloaded and further impairment of contractility may be avoided.

Previous studies have also shown that there is a progressive recruitment of expiratory muscles with increasing CO_2 (22,23,28) and during exercise (1,9,29) or loading (1,26,28,31). My results are consistent with these observations. In addition, I found a further decrease in end-expiratory P_L and an increase in end-expiratory Pga indicating a further decrease in end-expiratory lung volume after global inspiratory muscle fatigue (figure 6.5). When end-expiratory lung volume is below the equilibrium volume of the respiratory system, the initial part of inspiration is passive and driven by relaxation of expiratory muscles with conversion of elastic energy stored in the system into kinetic energy without

requiring any inspiratory muscle recruitment. This further diminishes the demands on the inspiratory muscles.

The partitioning of V_T into that contributed by expiratory muscle relaxation ($V_{T,E}$) and that contributed by inspiratory muscle contraction ($V_{T,I}$) can be estimated by assuming that lung compliance (C_L) is 0.2 litres/cmH_2O and that $V_{T,E}$ at the start of rebreathing before fatigue is zero. $V_{T,E}$ then is the product of C_L and ΔP_L where ΔP_L is the decrease in end-expiratory P_L as a function of $P_{ET}CO_2$ before and after fatigue. These calculations are shown in figure 6.6. $V_{T,I}$ is the difference between V_T and $V_{T,E}$ ($V_{T,I}=V_T-V_{T,E}$) and is illustrated by the vertical distance between V_T (open squares) and $V_{T,E}$ (closed aquares) of figure 6.6. According to this estimation, I found that $V_{T,E}$ increased linearly with increasing $P_{ET}CO_2$ and reached 0.58 and 0.96 litres at $P_{ET}CO_2$ 57.5 mmHg before and after global fatigue, respectively (25% and 55% of the corresponding V_T). The slope of the $V_{T,E}$, $P_{ET}CO_2$ relationship was the same before and after fatigue so that the additional $V_{T,E}$ after fatigue was about 0.38-0.46 litres irrespective of $P_{ET}CO_2$. Figure 6.6 also shows that after global inspiratory muscle fatigue $V_{T,I}$ (the distance between V_T and $V_{T,E}$) was significantly reduced as a result of both the decrease in total V_T and the increase in $V_{T,E}$.

In figure 6.7, $V_{T,I}$ along with the elastic work of inspiration (W_I) and inspiratory elastic power ($\dot{W}_I$) after global fatigue is expressed as a percentage of the value obtained before fatigue. The elastic work per breath performed by the inspiratory muscles above FRC is given by: $W_I = 1/2 \cdot V_{T,I} \cdot \Delta P_{rs}$ where ΔP_{rs} is the elastic

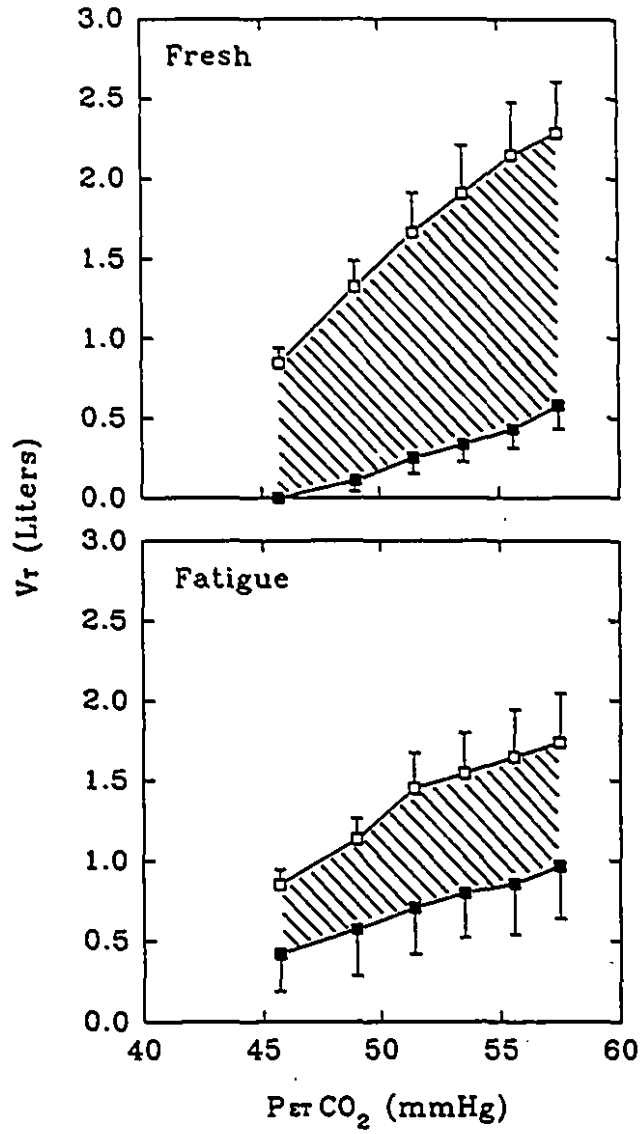

Figure 6.6: Tidal volume response to CO_2. V_T: tidal volume; $P_{ET}CO_2$: end-tidal partial pressure of CO_2 . Open squares: V_T measured by integration of flow as shown in figure 6.1; closed squares: V_T contributed by expiratory muscle recruitment ($V_{T,E}$). The vertical distance between V_T and $V_{T,E}$ represents the V_T contributed by inspiratory muscles ($V_{T,I}$). $V_{T,I}=V_T-V_{T,E}$.

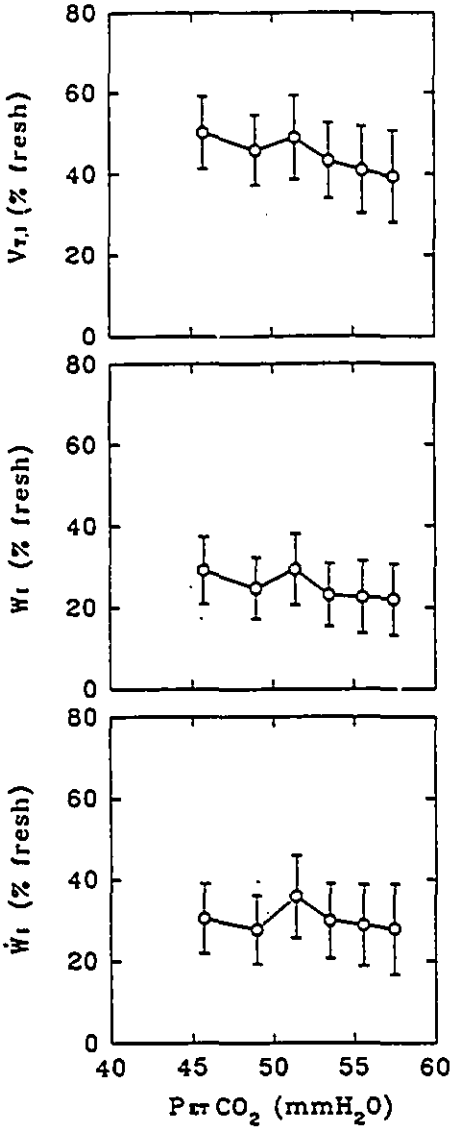

Figure 6.7: The V_T contributed by inspiratory muscles ($V_{T,I}$), the elastic work of inspiration performed by inspiratory muscles (W_I), and power ($\dot{W}_I$) after global inspiratory muscle fatigue expressed as a percentage of the value for the same $P_{ET}CO_2$ before fatigue.

pressure developed by the inspiratory muscles to inflate the respiratory system from FRC to FRC + $V_{T,I}$ (3). $\Delta P_{rs} = V_{T,I} \cdot E_{rs}$, where Ers is the elastance of the respiratory system. Substituting: $W_I = 1/2 \cdot V_{T,I}^2 \cdot E_{rs}$. Assuming that Ers is constant after fatigue, W_I after fatigue expressed as a fraction of that before fatigue is thus given by the ratio of $V_{T,I}^2$ after fatigue to that before. Similarly $\dot{W}_I$ after fatigue as a fraction of that fresh is the same ratio multiplied by the ratio of respiratory frequencies. Figure 6.7 shows that after global fatigue $V_{T,I}$, W_I, and $\dot{W}_I$ were below 50%, 30%, and 40% of the value obtained before fatigue, respectively, throughout the CO_2 rebreathing. Therefore, with the adaptation of breathing pattern to rapid shallow breathing and with expiratory muscle recruitment, $\dot{V}_E$ response to CO_2 can be maintained and the inspiratory muscles substantially unloaded in spite of significant decrease in inspiratory muscle contractility after global inspiratory muscle fatigue.

These calculations were based on the estimation of $V_{T,E}$, which was in turn dependent on assumptions that changes in end-expiratory P_L were due to expiratory muscle recruitment, that C_L was equal to 0.2 litres/cmH_2O and did not change with fatigue. A reduction in end-expiratory lung volume could result from an influence of global fatigue on chest wall compliance (C_W). Although C_W is not affected by diaphragmatic fatigue (40), it has not been measured after global inspiratory muscle fatigue. Changes in body posture and balloon position are possible after fatigue but would be unlikely to be systematic or cause such marked changes that I observed. In

spite of these uncertainties, I found that my subjects had a consistent shift of end-expiratory P_L-Pga relationship downwards and to the right with increasing CO_2 and after global inspiratory muscle fatigue. I therefore believe that most of these changes reflect expiratory muscle recruitment rather than other factors, and conclude that the strategy chosen to maintain $\dot{V}_E$ at any given level of $P_{ET}CO_2$ after glogal fatigue, markedly reduces the power output of the inspiratory muscles.

Animal studies have shown that severe decrement of respiratory muscle contractility rarely occurs in spite of the development of rapid shallow breathing when the animals breath against high inspiratory resistance (2,20). Thus, rapid shallow breathing with expiratory muscle recruitment to decrease end-expiratory lung volume may not be a manifestation of fatigue but a compensatory mechanism to avoid it. Although this strategy may be efficient for inspiratory muscle energetics it may not work in COPD patients. When airway resistance is increased and expiratory flow limitation is present, increasing f results in a decrease in expiratory duration leading to dynamic hyperinflation with an increase in end-expiratory P_L (19). Under these circumstances, it may be mechanically impossible to decrease end-expiratory lung volume and diminish W_I. Instead, end-expiratory lung volume will increase rather than decrease even with expiratory muscle recruitment; this will result in an increase in elastic work of inspiration. The effect of a fatiguing load in the presence of expiratory flow limitation is thus potentially disastrous.

In summary, global inspiratory muscle fatigue results in recruitment of expiratory muscles and derecruitment of inspiratory muscles leading to rapid shallow breathing. My results demonstrate remarkable plasticity of the ventilatory control system, to the extent that the drive to the respiratory muscles changes appropriately according to their contractile state in order to maintain the $\dot{V}_E$, $P_{ET}CO_2$ relationship which is highly conserved. How the plasticity of ventilatory drive is achieved is worthy of future research. However, even though $\dot{V}_E$ is highly conserved the development of rapid shallow breathing in order to minimize inspiratory muscle work and power will result in a decrease in alveolar ventilation and for a constant CO_2 production, $PaCO_2$ will increase. This may be an important mechanism in the development of hypercapnic respiratory failure.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The purpose of my present work has been to assess and evaluate human respiratory muscle fatigue. The first part composed of chapter 2 through chapter 4 evaluated an improved technique of bilateral supramaximal transcutaneous phrenic nerve stimulation as a diagnostic test to monitor diaphragmatic function and detect fatigue. The second part composed of chapter 5 and chapter 6 evaluated the influence of either diaphragmatic or global inspiratory muscle fatigue on ventilatory responses and respiratory muscle recruitment during CO_2 rebreathing.

The diagnosis of respiratory muscle fatigue remains a serious problem for respiratory physicians. Any bed-side diagnostic procedure requires that the technique be relatively simple and non-invasive, but reliable, objective, sensitive and specific. It has already been agreed that bilateral phrenic nerve stimulation is probably the best candidate for this purpose. However, this technique needed to be vigorously evaluated and improved before it can be recommended for routine diagnostic use. With this background using bilateral supramaximal transcutaneous phrenic nerve stimulation, I firstly determined the twitch transdiaphragmatic pressure (Pdi,T)-lung volume relationship and demonstrated that although the relationship remained linear before and after diaphragmatic fatigue task, there was a proportionately greater loss of diaphragmatic force at high lung volumes, suggesting that lung volume is an important independent variable for Pdi,T measurement which should be taken into consideration whenever the measurements are done. Secondly, I tested the possibility of

employing twitch mouth pressure (Pm,T) as a non-invasive index to assess diaphragmatic fatigue. I found that this is quite feasible; there was an inverse linear relationship between Pm,T and lung volume; after diaphragmatic fatigue Pm,T decreased in a similar proportion as did Pdi,T resulting in an unchanged Pm,T-Pdi,T relationship. Therefore, the changes in Pm,T for a given lung volume reflects the changes in Pdi,T and in turn reflects the changes in diaphragmatic contractility. Thirdly, I demonstrated that bilateral phrenic nerve paired shocks (with varied time intervals) could be used to measure force-frequency relationship of the diaphragm and detect diaphragmatic low frequency fatigue. Phrenic nerve paired shocks are better and more suitable than phrenic nerve tetanic stimulation as a diagnostic procedure since the former was well tolerated by my subjects and the latter was not.

For clinical diagnostic purpose bilateral supramaximal transcutaneous phrenic nerve stimulation is a relatively simple technique but which requires some training and practise. The elicited diaphragmatic twitches are reproducible and are easily accepted by most patients. As it requires little cooperation from the subjects or patients compared with voluntary manoeuvres, the technique provides an objective measure of diaphragmatic contractility.

Although respiratory muscle fatigue and control of breathing have been extensively studied and reporded in the literature, no attempts have been made to systematically investigate the effect of

respiratory muscle fatigue on control of breathing. It was unknown whether or not respiratory muscle fatigue modifies ventilation and its control.

In the present work in normal subjects with CO_2 rebreathing, I showed that there are substantial alterations in ventilatory response and respiratory muscle recruitment after fatigue. However, there are both qualitative and quantitative differences of the responses dependent on the fatigue protocol employed. Following diaphragmatic fatigue, the only change is a proportionately greater recruitment of inspiratory rib cage muscles, which compensates for the reduced diaphragmatic contribution to breathing, so that the ventilatory parameters remain constant. After global inspiratory muscle fatigue, the subjects develop rapid shallow breathing. Both the diaphragm and the inspiratory rib cage muscles contribute less but expiratory muscles contribute more to tidal volume. In spite of these changes, minute ventilation ($\dot{V}E$) at any given $PETCO_2$ remains constant after global fatigue.

These results suggest that brainstem respiratory control centres may detect and localize fatigue. Their command has sufficient plasticity, to the extent that they keep overall ventilation constant by decreasing drive to the fatigued respiratory muscles while increasing drive to those muscles that presumably remain fresh, If this fails to maintain tidal volume, breathing frequency increases in order to preserve $\dot{V}E$. How respiratory control system "sense" fatigue and respond to it approperiately according to muscle's contractile state so that $\dot{V}E$

can be conserved is beyond the scope of my present study, and deserves further investigations.

The following points can be added to the literature as original contributions of my present work to our understanding of respiratory muscle mechanics and fatigue:

1. Both before and after fatigue, Pdi,T is linearly and inversely related to lung volume. After diaphragmatic fatigue, the most clear-cut and long-lasting change is the significant decrease in the amplitude of Pdi,T at all lung volumes (between FRC and TLC) compared with other changes in Pdi,T such as rate of contraction and relaxation. Pdi,T-lung volume relationship is therefore useful to assess diaphragmatic contractility and fatigue.

2. After diaphragmatic fatigue, there is a proportionately greater decrease in Pdi,T amplitude with increasing lung volume. This volume-dependent additional decrease in Pdi,T recovers fast within 15 to 30 minutes. The overall recovery at all lung volumes is longer than 1 hour. Therefore, if this test is to be used diagnostically, there is a broad time period to employ before recovery occurs.

3. Diaphragmatic fatigue does not affect the relaxed compliance of the chest wall partitioned into the rib cage and abdomen. Likewise, it does not affect inspiratory function of the diaphragm expressed as the ratio $\Delta Pes/\Delta Pdi$ during a twitch.

4. In normal subjects, Pm,T is linearly related to twitch esophageal pressure (Pes,T) and to Pdi,T, and inversely and linearly related to lung volume. These relationships are

reproducible on separate days for a given subject.

5. The slope of Pm,T-Pes,T relationship which is slightly different from 1.0 in normal subjects is not due to airway time constant, but most probably attributable to pleural pressure gradients potentiated by isolated diaphragmatic twitch contraction.

6. After diaphragmatic fatigue, the amplitude of Pm,T decreased at all lung volumes in proportion to the decrease in Pdi,T. The Pm,T-Pes,T and Pm,T-Pdi,T relationships remain unaltered. These results indicate that Pm,T-lung volume relationship can be employed to detect diaphragmatic fatigue non-invasively.

7. The pressure-frequency relationship of the diaphragm can be established with phrenic nerve paired shocks. The shape of the relationship thus obtained plateaued at lower stimulation frequencies and thus differs from that obtained with tetanus. The difference results from the fewer number of stimuli delivered. However, the pressure-frequency curves of the diaphragm with paired shocks are quite reproducible for a given subject on separate occasions, and after diaphragmatic fatigue, ΔPdi significantly diminishes at all stimulation frequencies of paired shocks.

8. In the fresh state, the amplitude of diaphragmatic twitch in response to the second shock (T2) is progressively decreased with increasing stimulation frequency with the amplitude ratio at 10Hz over that at 100Hz ($T2_{10/100}$) significantly greater than 1.0. After diaphragmatic fatigue, $T2_{10/100}$ becomes substantially smaller than or close to 1.0. In addition, the time course of $T2_{10/100}$

follows that of low frequency fatigue. These data suggest that phrenic nerve paired shocks may replace tetanic stimulation and $T2_{10/100}$ may be a reliable parameter for low frequency fatigue.

9. There is a substantial alteration of the respiratory muscle recruitment during CO_2 rebreathing after respiratory muscle fatigue. As a result ventilatory pattern can be well preserved or changed dependent on the fatigue pattern.

10. After diaphragmatic fatigue, none of the ventilatory parameters including tidal volume (V_T), breathing frequency (f), minute ventilation ($\dot{V}_E$), duty cycle (Ti/Ttot), and mean inspiratory flow (V_T/Ti) change; after global inspiratory muscle fatigue, there is a decrease in V_T response and an increase in f response to CO_2 referred to as rapid shallow breathing, $\dot{V}_E$, Ti/Ttot, and V_T/Ti remain constant.

11. After diaphragmatic fatigue, there is a proportionately greater increase in the recruitment of inspiratory rib cage muscles and diaphragmatic contribution to breathing is diminished; after global fatigue, both the diaphragm and inspiratory rib cage muscles reduce their contribution to breathing.

12. Diaphragmatic fatigue does not change expiratory muscle recruitment since increased rib cage muscle recruitment fully compensates for the decreased diaphragmatic contractility and conserves all ventilatory parameters constant; global fatigue further recruits expiratory muscles and decreases end expiratory lung volume storing elastic energy in the respiratory system. This stored energy performs the initial work of inspiration which is

accomplished without inspiratory muscle recruitment. The resulting volume change is estimated to be around 0.4 litres.

13. As global fatigue reduces V_T and increases expiratory muscle contribution to V_T, the V_T generated by inspiratory muscles ($V_{T,I}$), the elastic work (W_I) and power ($\dot{W}_I$) of inspiration are significantly reduced. $V_{T,I}$, W_I, and $\dot{W}_I$ do not change after diaphragmatic fatigue because V_T and expiratory muscle recruitment do not change, though diaphragmatic contribution to V_T is decreased, while that of the rib cage muscles is increased.

14. The results of my work suggest that respiratory control system is able to detect fatigue and respond appropriately to it according to each muscle's contractile state in order to maintain $\dot{V}_E$ constant but not to drive the fatigued respiratory muscles to exhaustion. However, the rapid shallow breathing thus developed may lead to alveolar hypoventilation and hypercapnic respiratory failure.

APPENDIX 1: S.I. Unit Equivalents

1 newton/cm^2	10000 Pa
1 cmH_2O	98 Pa
1 mmHg	133 Pa
1 atmospheric pressure	101,325 Pa

Appendix 2: Abbreviations

AB	abdomen
ANOVA	analysis of variance
AP	anterior-posterior
APab	anterior-posterior dimension of the abdomen
APrc	anterior-posterior dimension of the rib cage
a.u.	arbitrary unit
°C	degrees centigrade
C_L	lung compliance
cm	centimeter
cmH_2O	centimeter water colume
CO_2	carbon dioxid
COPD	chronic obstructive pulmonary disease
C'rc	effective compliance of the rib cage
CT	contraction time
CV	coefficient variation
C_W	chest wall compliance
ECG	electrocardiogram
Edi	diaphragmatic electromyogram
EMG	electromyogram
Ers	elastance of respiratory system
f	breathing frequency
FG	fast twitch glycolytic
Fig	figure
GLM-ANOVA	general linear model analysis of variance or covariance
FOG	fast twitch oxidative glycolytic
FRC	functional residual capacity
H	hour
Hb	haemoglobin
H/L	high-to-low frequency ratio
Hz	Hertz
L	Litre
IC	inspiratory capacity
IP	intraperitoneal
L_o	muscle optimal length
M1	the first M-wave
M2	the second M-wave
min	minute
mM	millimolar
mmHg	millimeter mercury colume
MRR	maximal relaxation rate
ms	millisecond

M-wave	Muscle mass action potential
n	number
N/cm^2	newton per suqare centimeter
O_2	oxyzen
$P_{10/100}$	force ratio at 10Hz over that at 100Hz
P_{10}	tetanic force at 10Hz
P_{100}	tetanic force at 100Hz
Pa_{10}	paired twitches at 10Hz
Pa_{100}	paired twitches at 100Hz
Pab	abdominal pressure
$PaCO_2$	arterial carbon dioxide partial pressure
Pdi	trandiaphragmatic pressure
Pdi,max	maximal trandiaphragmatic pressure
Pdi,s	transdiaphragmatic pressure swing
$Pdi,s_{10/100}$	trandiaphragmatic pressure swing ratio at 10Hz over that at 100Hz
Pdi,T	twitch transdiaphragmatic pressure
Pes	esophageal pressure
Pes,T	twitch esophageal pressure
$PETCO_2$	end-tidal carbon dioxid partial pressure
Pga	gastric pressure
Pga,T	twitch gastric pressure
PI,max	maximal inspiratory pressure
P_L	transpulmonary pressure
Pm	mouth pressure
Pm,max	maximal mouth pressure
Pm,T	twitch mouth pressure
Pnp	nasopharyngeal pressure
Po	maximal isometric force
Ppl	pleural pressure
Ppl,max	maximal pleural pressure
Prs	respiratory system pressure
P_t	single twitch tension
Ptr	tracheal pressure
Ptr,T	twitch tracheal pressure
τ	time constant
RC	rib cage
1/2RT	half relaxation time
RV	residual volume
s	second
SaO_2	arterial oxyzen saturation
SD	standard deviation
SE	standard error
sec.	second
SO	slow twitch oxidative
T1	the first twitch

T2	the second twitch
$T2_{10/100}$	the second twitch ratio at 10Hz over that at 100Hz
T_F	time constant for fatigue
Ti	inspiratory time
TLC	total lung capacity
Tlim	endurance time limit
TTdi	tension-time index of the diaphragm
Ttot	total respiratory cycle time
TTrc	tension-time index of rib cage muscle
µm	micrometer
$\dot{V}_E$	minute ventilation
V_L	lung volume
V_T	tidal volume
$V_{T,E}$	tidal volume contributed by expiratory muscles
$V_{T,I}$	tidal volume contributed by inspiratory muscles
V_T/Ti	mean inspiratory flow
W_I	elastic work of inspiration
$\dot{W}_I$	elastic power of inspiration
wk	week
yr	year

APPENDIX 3: Academic Research Record

Publications

Papers

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Scientific meetings attended (with slides or poster presentation):

-American Thoracic Society Annual Meeting, Cincinnati, OH, 1989.

-American Thoracic Society Annual Meeting, Boston, MA, 1990.

-FASEB Annual Meeting, Washonton, Washington D.C., 1990.

-American Thoracic Society Annual Meeting, Anaheim, CA, 1991.

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