PROPOSED RESEARCH

The maximum length of this file is **10 pages** (letter size, Verdana size 10 is suggested). For an adequate evaluation of your proposal merits, this file must include the following aspects: Proposal description, Hypothesis, Goals, Methodology, Work Plan, Work in progress and Available Resources.

Keep in mind the Bases del Concurso de Proyectos FONDECYT Iniciación 2016 and Application Instructions.

PROPOSAL DESCRIPTION

Parkinson's disease

Parkinson´s disease (PD) is a slowly progressive neurodegenerative disorder characterised by motor and non-motor symptoms [1-3]. Motor symptoms of PD are associated with early death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of abnormal aggregates of α-synuclein protein called Lewy bodies and Lewy neuritis [2]. Classical motor features of the disease include bradykinesia, muscular rigidity, resting tremor, postural and gait impairment [2, 4, 5]. Non-motor symptoms are associated with non-dopaminergic cell dysfunction; however, the origin of neuroanatomical and neurochemical dysfunction is unknown [3]. The most disabling non-motor symptoms are falls, sleep disturbances, memory failure, confusional episodes, and dribbling of saliva [3]. Non-motor symptoms are correlated with advanced age and disease severity, and some of them such as olfactory problems, constipation, depression, and rapid eye movement disorder precede motor features of PD [2]. In PD there are multiple neuroanatomical areas affected outside the basal ganglia and although the cause of the disease is not known, there are genetic and environmental factors that contribute to develop the disease [2].

Parkinson´s disease is the second most common neurodegenerative disorder after Alzheimer´s disease [2, 6]. In South America the prevalence of PD is 31-470 per 100.000. The median age at onset is 60 years [1]; age is the greatest risk factor for developing PD [1, 2]. The incidence (in 100.000 person-years) rises from 17.4 between 50 and 59 years of age to 93.1 between 70 and 79 years [1]. Other risk factors are gender (male to female ratio 3:2) and ethnicity (highest in people of Hispanic ethnic origin)[2]. In Chile there are no accurate data regarding the prevalence of PD; however, it is estimated that from 1 to 2% of the population older than 65 years is affected by PD. This proportion rises in people older than 85 years to 3%-5% [7]. Given the longer life expectancy and the growing number of adults older than 60 years in Chile, it is expected an increase in the prevalence and mortality rate of PD. Therefore the introduction of new therapeutic modalities to treat the motor and non-motor symptoms of the disease, are becoming relevant to improve the quality of life of patients with PD as they live longer.

Respiratory problems in patients with Parkinson´s disease

Respiratory problems are common features in patients with PD [5, 8] and in particular, aspiration pneumonia is the leading cause of death [5, 8, 9]. Respiratory symptoms are usually asymptomatic until later stages of the disease, probably because restricted mobility does not lead to stress the respiratory system to become respiratory symptoms evident. [4, 8, 10] Previous studies regarding pulmonary function have reported that PD patients have upper airway obstruction; reduced peak inspiratory and expiratory flows, reduced maximum inspiratory and expiratory pressures, disturbances of ventilation and breathing patterns, electromyographic abnormalities of laryngeal muscles [4, 5, 8, 10], lower maximum voluntary ventilation [4], and impairment in the performance of rapid and coordinated contraction of limb and respiratory muscles during maximal efforts [4, 10]. These dysfunctions have been attributed to respiratory muscle weakness [5, 8], rigidity [5], akinesia, bradykinesia and hipokinesia [5, 8], stiffness of the chest wall [10], antiparkinsonian therapy [10] and reduced neural drive to respiratory muscles [5].

Studies about muscle function in patients with PD have shown that strength of limbs and respiratory muscles increases after antiparkinsonian medication [10, 11]. De Bruin et al [10] reported that 10 patients with PD significantly improved maximum inspiratory and expiratory pressures and maximum inspiratory and expiratory flows after medication with a dopamine agonist. Similarly, Corcos et al. [11] showed that removal of L-dopa therapy caused a decrease in isometric strength, rate of force development and time to

relaxation after contraction of elbow extensors. These findings suggest that muscle weakness is unlikely to be explained by peripheral changes in muscles and or nerves because antiparkinsonian medication is not known to affect peripheral neuromuscular function. Muscle weakness in PD is then attributed to an impaired central activation of motor units [5, 11]. Precisely the accumulating evidence suggests that strength training is feasible and useful for improving strength in patients with PD [12, 13]. The results of systematic reviews and metanalysis have revealed the positive effect of strength training on limb strength in patients with PD [14-16].

The high incidence of aspiration pneumonia in patients with PD has been attributed to disturbances in swallowing and respiratory function [5, 17-22]. Swallowing dysfunction or dysphagia is a common finding in patients with PD [21, 23] and it is present in early stages of the disease. Dysphagia can reach an incidence of up to 95% at later stages of PD[23]. Breathing and swallowing are highly coordinated motor functions controlled by the central nervous system. Both functions also share neuronal pathways and pharyngeal structures [18]. A close coordination between breathing and swallowing is necessary to transfer a bolus from the oral cavity into the lower digestive tract without penetration of the bolus into the airway [19]. This coordination is evident when a normal apnoea occurs during swallowing to protect the airway [18] Moreover, the presence of dysphagia in people with PD is associated with disordered cough function. Pitts et al. 2008 reported that patients with PD and evidence of penetration/aspiration, showed reduced voluntary cough airflow than patients without evidence of penetration/aspiration. Wheeler-Hegland et al. 2014, observed that patients with PD and dysphagia showed reduced sequential coughs in comparison to PD patients without dysphagia. This evidence suggests that disordered voluntary cough function may be an indicator of risk of penetration/aspiration in patients with PD and dysphagia.

Cough Function

Cough is a protective mechanism that removes foreign material from the airway by generating high expiratory flow [17, 20-22] An effective cough comprises the ability to expel gas at high linear velocity through the airway and an effective interaction between the flowing gas and mucus layer [24] A cough manoeuvre can be divided in three phases: inspiratory, compressive, and expiratory. In the inspiratory phase, there is a rapid inhalation of gas caused by contraction of the diaphragm and accessory respiratory muscles. The volume of gas inhaled varies from 50% of tidal volume to 50% of vital capacity. Inhalation of larger volume of gas will produce greater lengthening and optimize length-tension relationship of expiratory muscles. Then for a given degree of neural activation, inhaling to high lung volume will lead the expiratory muscles to produce greater positive intrathoracic pressure and greater expiratory flow and velocity [24]. In the compressive phase, the glottis closes and the isometric contraction of the expiratory muscles produces positive intra-abdominal and intrathoracic pressures. In the expiratory phase, the glottis opens abruptly and the intrathoracic pressure built in the compressive phase of cough produces high expiratory flow rates [24, 25].

Cough Function in patients with Parkinson´s disease

Studies about cough function have shown that patients with PD have reduced cough efficacy. This has been attributed to weakness and rigidity of respiratory muscles, bradykinesia of abdominal muscles, decrease cough reflex sensitivity, difficulty with coordinated contraction of upper airway and chest wall muscles [5, 10, 17, 20-22] These abnormalities have shown to be inversely correlated to the level of clinical disability. [5, 22] Ebihara et al [22] found that at early stages of the disease, the motor component of cough (i.e. peak cough flow) is impaired while in advanced stages of the disease both motor and sensory (i.e. cough reflex sensitivity) components of cough are impaired. Besides, in patients with PD at risk of aspiration or penetration of material into the airway, it has been reported a decreased voluntary cough airflow in comparison with PD patients without such a risk. [17, 20, 21] To date, it is not clear the relevance of each cough phase (i.e inspiratory, compressive or expiratory) in the amount of expiratory flow produced during an active or induced cough in patients with PD. Besides, the relationship between peak cough flow and pulmonary function parameters is not established yet, and which pulmonary function parameter should be improved to increase peak cough flow in patients with PD is unknown.

Respiratory muscle training

Given that reduced cough function is associated with decreased respiratory muscle strength, there is a need for strategies to treat cough dysfunction by exercising respiratory muscles. The evidence that

supports the use of respiratory muscle training among different healthy and patient populations is the same as that obtained from studying the effects of strength training of the limbs. [26-29] The abdominals, diaphragm and internal and external intercostal muscles have many of the same structural and metabolic properties as limb muscles, such as an equal distribution of type I and type II fibers [28-30]. These similarities make it possible to apply general training principles to respiratory muscle training. Respiratory muscle training programs have been utilized in patients with Parkinson´s disease [18, 27, 31, 32] and other similar neurodegenerative disorders such as amyotrophic lateral sclerosis,[33] multiple sclerosis [34-38] and Huntington´s disease [39] to improve pulmonary and swallowing function as well as exercise capacity. The results of an inspiratory muscle training study in patients with PD showed improvements in inspiratory muscle strength and endurance and a decrease in the perception of dyspnoea [31]. Similarly, studies regarding expiratory muscle training in PD have shown to produce a significant improvement in expiratory muscle strength,[27, 32] and cough volume acceleration measured by an airflow waveform produced during voluntary cough [32]. However, the number of these studies and their quality are insufficient to conclude whether peak cough flow improves after an inspiratory and/or expiratory muscle training program in patients with PD. [40]. In patients with PD, strengthening inspiratory muscles may lead to increase the volume of air inhaled during the inspiratory phase of cough, therefore a more effective cough will be possible by increasing the volume and flow of air exhaled during the expiratory phase of cough. Similarly, strengthening expiratory muscles will contribute to cough efficacy by increasing intrathoracic pressure, which promotes dynamic airway compression and augments exhaled gas velocity [24, 41, 42].

Air stacking

Given the role of the inspiratory phase in cough effectiveness, it seems possible to improve peak cough flow by increasing maximum insufflation capacity, which in turn can be increased by stacking air behind the glottis using a manual resuscitator bag [43]. The elastic recoil tension developed by lung tissue and chest wall expansion after maximum insufflation capacity can be used efficiently to produce an adequate flow cough during spontaneous cough manoeuvres.[25] The maximum amount of air that can be stacked depends on chest wall compliance and the effectiveness of oropharyngeal and laryngeal muscles in holding air behind the glottis. [43, 44] In patients with PD, respiratory muscle weakness, rigidity and chest wall stiffness may lead to reduced chest wall compliance and range of motion which in turn would reduce maximum insufflation capacity and cough flow. To the best of our knowledge, no previous studies have investigated the effects of air stacking alone or in combination with respiratory muscle training on peak cough flow in patients with PD. Therefore, given the relationship between maximum inspiratory capacity and respiratory muscle strength on cough phases[45], it is important to determine the effect of both interventions on peak cough flow in patients with PD. The air stacking technique has shown a positive effect in increasing maximum inspiratory capacity and peak cough flow in patients with neuromuscular diseases [44, 46, 47]. Therefore it seems plausible to obtain similar benefits of the air-stacking in patients with PD. It is hypothesized that air-stacking combined with respiratory muscle training would have larger effect on peak cough flow than each intervention only in patients with PD.

In summary pulmonary function is affected in patients with PD and this abnormality contributes to increase the risk of aspiration pneumonia, which is the leading cause of death. Cough is an important defence mechanism that removes foreign material from the airway and prevents an aspiration. However patients with PD have shown reduced cough efficacy that is associated with decreased respiratory muscle strength and reduced airflow during the expiratory phase of cough. Therefore there is a need of strategies aimed at improving cough efficacy and reduce the risk of aspiration pneumonia. It is possible therefore, that improving cough efficacy in patients with PD will impact on their quality of life and prolong their survival time.

AIMS AND HYPOTHESIS

General Aims

The general aims of this project are:

1. To determine which pulmonary function parameter is associated with reduced peak cough flow in patients with PD.
2. To determine which pulmonary function parameter is more relevant to improve peak cough flow in patients with PD.

3. To compare the effects of an inspiratory muscle-training program on peak cough flow and pulmonary function in patients with PD in comparison to an expiratory muscle-training program.
4. To investigate the effects of air stacking in addition to an inspiratory and an expiratory-muscle training program to increase maximum inspiratory capacity, pulmonary function, and peak cough flow in patients with PD.
5. To determine the impact of an increased voluntary cough airflow on the risk of penetration/aspiration, in patients with Parkinson´s disease.

General Hypothesis

The general hypotheses of this project are:

1. Pulmonary function parameters such as maximum inspiratory and expiratory pressures would show a direct and proportional relationship with voluntary and induced peak cough flow. Therefore improvements in maximum respiratory pressures would be more relevant to produce positive changes in peak cough flow in patients with PD.
2. An expiratory muscle-training program would be more effective than an inspiratory muscle-training program to improve peak cough flow in patients with PD.
3. The use of air stacking and an expiratory muscle training program would be more effective than the use of air stacking and an inspiratory muscle training program to improve maximum inspiratory capacity and peak cough flow in patients with PD
4. In patients with Parkinson´s disease and dysphagia, an increase in peak cough flow after expiratory muscle training and air stacking, would be associated with a lower score in the penetration/aspiration score.

To achieve these aims, this project will allocate participants into five treatment-groups. In group 1, participants will be assigned to receive home-based inspiratory muscle training; in group 2 participants will perform home-based expiratory muscle training, in group 3 participants will be assigned to receive home-based expiratory muscle training at minimum and fixed load (control group). In group 4 participants will be assigned to receive home-based inspiratory muscle training and air stacking and finally in group 5 participants will perform home-based expiratory muscle training and air stacking. For practical purposes this project will include two studies. Study 1 will compare the effects of an inspiratory muscle-training program versus an expiratory muscle-training program on peak cough flow and pulmonary function in patients with PD. Baseline measurements of study 1 will be used to determine the relationship between peak cough flow and pulmonary function parameters and to define which pulmonary function parameter would be more relevant to improve peak cough flow in patients with PD. Study 2 will investigate the effects of air stacking in addition to an inspiratory and an expiratory-muscle training program to increase maximum inspiratory capacity, pulmonary function, and peak cough flow in patients with PD (Figure1).

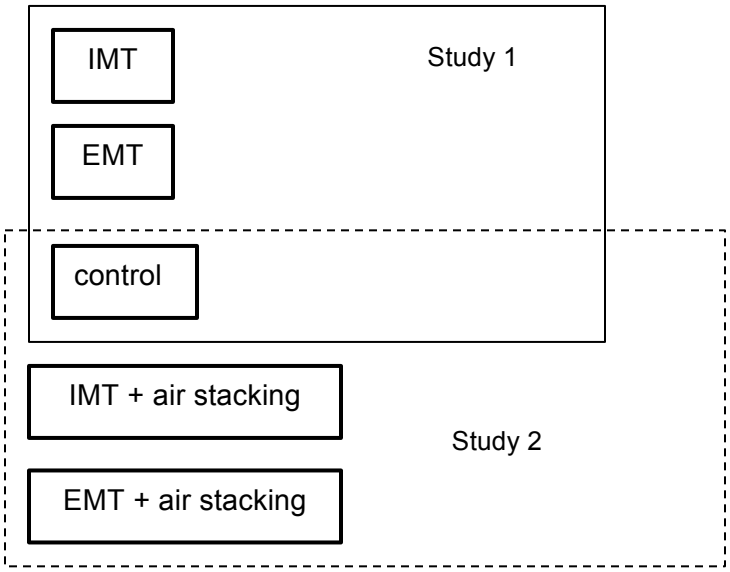

Figure 1. A schematic representation of study groups for study 1 and 2.

METHODOLOGY

Study 1
Participants

Patients of both genders with diagnose of idiopathic Parkinson´s disease and moderate level of disability (stage II and III Hoehn and Yahr scale) will be recruited in this study. Inclusion criteria will be: 1) diagnosis of idiopathic Parkinson´s disease confirmed by a neurologist; 2) patients with the ability to understand and respond to the instructions given in the study; 3) unaltered dose and type of antiparkinsonian medication and 4) stable disease at the moment of inclusion in this study. Exclusion criteria will be: 1) other concomitant neurological disease; 2) current smokers; 4) participants with a history of cardiovascular pathology, lung disease or the presence of respiratory symptoms such as cough, phlegm, wheezing or dyspnoea at the time of assessment. Participants with difficulties in maintaining a proper mouth seal or unable to avoid air leakage during pulmonary function testing will be also excluded. Informed written consent will be obtained from all participants in the study. The study protocol will be submitted to ethical committee of Pontificia Universidad Catolica de Chile. This study will employ a 3 (groups) x 3 (measure times: Baseline, 1 and 2 months) repeated measures design. Sample size was estimated based on the result of the study by Sapienza et al. 2011 [27] in which patients with PD underwent an expiratory muscle training program and peak expiratory flow was compared between an experimental and a control group. Using an alpha level of 0.05, a statistical power of 0.8 and an estimated improvement of at least 1.5 (L/s) in peak expiratory flow (SD= 1.67); after 2 months of training in the expiratory muscle training group, it was estimated a sample size of 13 participants per group.

Experimental Design

All participants of the study will be interviewed regarding their general health status, cardiovascular and smoking history as well as their respiratory condition. The body mass and height will be recorded for each participant using an accurate and calibrated scale and a wall-mounted stadiometer. After baseline measurements, participants will be divided into three groups using the minimization method (Scott et al, 2002). Given that 39 participants will be recruited, 13 participants will be assigned to receive home-based inspiratory muscle training, 13 participants will be assigned to receive home-based expiratory muscle training, and 13 participants will be assigned to receive home-based expiratory muscle training at minimum and fixed load (control group).

Training Protocol

Participants in the inspiratory training group will perform a home-based inspiratory (5 sets of 5 repetitions) muscle-training program using a Threshold® Inspiratory Muscle Trainer (HS730-010. Phillips Respironics, USA). Participants in the expiratory training group will perform a home-based expiratory (5 sets of 5 repetitions) muscle-training program using an Expiratory Muscle Trainer (EMST150. Aspire Products, LLC). Both groups will train 6 times a week for 2 months. These training parameters are chosen because previous studies have shown significant improvements in pulmonary function in patients with Parkinson's disease using a similar protocol[40]. Participants in both groups will train at a resistance equal to 75% of their average maximum inspiratory pressure and maximum expiratory pressure, which will be calculated based on maximum inspiratory pressure and maximum expiratory pressure baseline measurements described below. The resistance will be adjusted every two weeks.
Participants in the control group will use a Threshold® PEP (HS-735-010. Phillips Respironics, USA) for expiratory muscle training because this device provides lower resistance for expiratory flows than those devices used by the training groups. They will train using the same protocol of the participants in the training groups for the same number of repetitions, frequency and duration, but the intensity will be fixed at the minimum load of the device, 9 centimetres of water throughout the training period.
Before the commencement of the training, participants in both groups will be instructed for the correct use of the training devices. For inspiratory muscle training, they will be instructed to initiate their inspiration from near to residual volume, while for expiratory muscle training, they will be asked to blow as forcefully as possible into the mouthpiece of the trainer device from total lung capacity. In both cases participants will use a nose clip to ensure that airflow occurs entirely through the mouth. All participants will be asked to mark on the provided training diary when a training session is completed. All participants will be cited every two weeks, to ensure that they will be training as instructed and to reassess maximum inspiratory and expiratory pressures. Daily phone contacts or whatsapp messages from Monday to Friday throughout

the study period will be sent to remind participants to perform the training as instructed. In this study we will use the pressure threshold modality in which the load valve of the training device remains closed until enough inspiratory or expiratory pressure is generated against the valve to release it and allow airflow. Therefore maximum inspiratory and expiratory pressure measurements will be used to adjust the resistance of the devices every two weeks for the training groups.

Measurements

Spirometric indices, maximum inspiratory pressure, maximum expiratory pressure, peak expiratory flow during reflex and voluntary cough and the degree of penetration/aspiration on a swallowing task during a video-fluoroscopy will be taken during three weeks before the commencement of the training to obtain baseline assessment. Measurements of maximum inspiratory pressure and maximum expiratory pressure will be performed over 3 testing sessions with a week apart to assess the test-retest reliability. Spirometry, maximum inspiratory pressure and maximum expiratory pressure will be repeated at 1 and 2 months after training. Peak expiratory flow (reflex and voluntary cough) and video-fluorographic analysis of swallowing will be repeated at 2 months after training. All measurements will be performed at the same place and same time of the day. To minimize possible acute effects of the training session on the measures, 1 or 2 days will be inserted between the training session and the measurements.

Maximum inspiratory and expiratory pressure

For maximum inspiratory pressure and maximum expiratory pressure measurements, each participant will be asked to sit upright with a nose clip in place to prevent nasal air leakage. A flanged rubber mouthpiece will be connected to a pressure manometer (Micro RPM, Micro Medical-Care Fusion, Kent, UK) and placed in the mouth. Participants will be asked to hold the pressure manometer with both hands and to create a tight lip seal around the flanged mouthpiece. A flanged mouthpiece will be used as recommended by the American Thoracic Society and European Respiratory Society [48]. This method will be used, since the portability of the manometer was advantageous for clinical use and its reliability had been assessed[49]. For maximum expiratory pressure assessment, participants will be asked to breathe in to total lung capacity and then to blow hard into the mouthpiece. For maximum inspiratory pressure assessment, they will be instructed to breath out to residual volume and then to breath in with maximum effort through the mouthpiece. Inspiratory and expiratory efforts will be required to be maintained for more than one second. The order of procedures will be the inspiratory followed by the expiratory effort. Both manoeuvres will be repeated a minimum of 5 times with a 30-s rest until three trials show values within 5% variation of each other. The best result from at least five respiratory manoeuvres will be used for analysis as described by Black and Hyatt (1969) and the professional society guidelines. Results of maximum inspiratory pressure and maximum expiratory pressure will be expressed as absolute and relative values using Black and Hyatt reference values [50].

Spirometry

Spirometric assessment will be performed using a portable spirometer (Micro Lab^{TM}). The spirometer meet all the quality control requirements of the American Thoracic Society and will be calibrated before each testing session with a Hans Rudolph 3.0 syringe, based on the manufacturer's recommendations. In accordance with the professional society guidelines, each participant will be asked to sit upright with a nose clip in place to prevent nasal air leakage. Participants will be instructed to perform spirometric maneuvers in the following order: slow vital capacity, a forced vital capacity and maximal voluntary ventilation. Each manoeuvre will be repeated at least 3 and a maximum of 8 times, with 1–2 min rest between attempts. The best result from the three technically acceptable manoeuvres will be used for analysis. From these measurements, forced expiratory volume in one second, peak expiratory flow and the ratio of forced expiratory volume in one second to forced vital capacity from the largest forced expiratory volume in one second and forced vital capacity will be calculated. Predicted values will be calculated for all participants using Gutierrez et al. 1996 prediction equations, which include age, height, weight and gender. These prediction equations include reference values for forced vital capacity and forced expiratory volume in one second, and have been validated for Chilean population [51].

Cough flow

For reflex cough airflow measurement patients will be asked to sit upright and wear a facemask covering the nose and mouth. The facemask will be coupled to a pneumotachograph that input differential pressure change to a digital spirometer. A delivery port with a one-way inspiratory valve for nebulization will be connected to the facemask. The nebulizer will be connected to a dosimeter that will deliver aerosolized solution during inspiration for 2s. Cough will be induced using capsaicin dissolved in a solution consisting of

80% physiological saline, and 20% ethanol. The capsaicin solution will be diluted in a single dose of 200um. The vehicle solution alone (80% physiological saline, 20% ethanol) will be administered as a control aerosol. Patients will initiate the testing with 30 s of tidal breathing to acclimate to the facemask. Before the capsaicin solution is delivered, patients will be told to "cough if you need to cough into the facemask". The capsaicin solution will be automatically administered when the beginning of the inspiratory phase of tidal breathing is detected. The capsaicin solution and the vehicle solution will be administered randomly to avoid anticipation from participants. Participants will be instructed to keep breathing as the solution is delivered. Airflow will be recorded until at least 2 coughs were produced, or for up to 30s following the presentation of the capsaicin solution.
For voluntary cough airflow measurement, participants will be connected to the same pneumotachograph used previously, however the nebulizer will not contain any solution. Participants will be asked to "cough as if something went down to the wrong pipe". Participants will perform first voluntary cough assessment followed by reflex cough to avoid any residual sensation of capsaicin [17, 52, 53].

Video-fluorographic measurement of swallow
Participants will be asked to sit upright and swallow 90 ml of a thin liquid mixed with a contrast solution (barium sulphate) in a continuous manner. A specialist will rate the degree of penetration/aspiration from the video-fluorographic images using the penetration/aspiration ordinal scale [54].

Statistical Analysis
Descriptive data will be given as mean, standard deviation and range. To compare physical and physiological characteristics and baseline measurements between training and control groups, an independent t-test will be performed. To assess the test-retest reliability of maximum inspiratory pressure and maximum expiratory pressure measurements, an interclass correlation coefficient (ICC) and the coefficient of variation (CV) will be calculated for each group (training and control). The level of linear relationship between peak cough flow and pulmonary function parameters will be determined using Pearson correlation coefficient. Normality assumption will be tested using the Shapiro-Wilk test. Changes in pulmonary function parameters due to group and or time effect will be tested using repeated measures ANOVA. Changes in the penetration/aspiration scale will be analysed using Friedman´s analysis of variance test and the Wilcoxon signed rank sum test. Statistical significance will be set at $P \leq 0.05$. Statistical analyses will be performed using STATA statistical software version 9.1

Study 2
Participants
Patients of both genders with diagnose of idiopathic Parkinson´s disease and moderate level of disability will be recruited in study 2. Same inclusion and exclusion criteria of study 1 will be applied in study 2. In this second study, groups will be as follows: Patients that performed expiratory muscle training at low intensity (control group in study 1) will perform home-based expiratory muscle training and air stacking. A new group of patients with PD that did not participate in study 1 will be recruited and perform home-based inspiratory muscle training and air stacking. Patients, who performed inspiratory muscle training and expiratory muscle training in study 1, will be included in study 2 for the purpose of the analysis. Study 2 will employ a 4 (groups) x 2 (measure times: Baseline and 2 months) repeated measures design. To the best of our knowledge there are no studies regarding the effects of respiratory muscle training and air stacking in patients with PD. Studies about techniques to enhance peak cough flow in patients with neuromuscular diseases, provide the best approximation possible to estimate a sample size. For a repeated measure ANOVA design using an alpha level of 0.05, a statistical power of 0.8 and an estimated effect size of at least 0.6 in peak expiratory flow after 2 months of expiratory muscle training and air stacking, it is estimated a sample size of 13 participants per group.

Experimental Design
Same interview and baseline measurements of study1 will be applied for all new participants recruited in study 2. After baseline measurements, participants will be allocated into these following groups: 13 patients will be assigned to perform home-based inspiratory muscle training and air stacking and 13 patients will be assigned to perform home-based expiratory muscle training and air stacking.

Training Protocol
Participants in the inspiratory muscle training and air-stacking group will perform home-based inspiratory muscle training using the same device and training protocol of the participants in study 1 for the same number of repetitions, frequency, duration and intensity. The air-stacking program will consist of 10 series

of three to four consecutive lung insufflations by air-stacking 6 times a week for two months. Participants in the expiratory muscle training and air-stacking group will perform home-based expiratory muscle training using the same device and training protocol of the participants in study 1 for the same number of repetitions, frequency, duration and intensity. The air-stacking program will be the same described above. All participants will perform first air-stacking followed by inspiratory or expiratory muscle training. This air-stacking program was chosen because previous studies have shown significant improvements in unassisted and assisted peak cough flow in patients with neuromuscular diseases [44, 46, 47]. The resistance of the inspiratory and expiratory muscle-training device will be adjusted every two weeks. Before the commencement of the training, participants in both groups will be instructed for the correct use of the training devices and the manual resuscitator bag. For inspiratory and expiratory muscle training participants will receive the same instructions described above in study 1. All patients of both groups will receive a manual resuscitator attached to a facemask. Each patient and his/her respective caregiver will be trained in the air-stacking technique. They will be instructed to perform the insufflations with the patient in a sitting position and the caregiver positioned behind the patient. The maximum inspiratory capacity by air stacking will be achieved by the patient taking a deep breath, holding it, and then air-stacking consecutively to the maximum volume that could be held with closed glottis. Participants will be instructed to hold the total volume of air for as much time as possible and then exhale. All participants will be asked to mark on the provided training diary when a session is completed. All participants will be cited every two weeks, to ensure that they will be training as instructed and to reassess maximum inspiratory and expiratory pressures. Daily phone contacts or whatsapp messages from Monday to Friday throughout the study period will be sent to remind participants to perform the training as instructed.

Measurements

Spirometric indices, maximum inspiratory pressure, maximum expiratory pressure, peak expiratory flow during reflex and voluntary cough and the degree of penetration/aspiration on a swallowing task during a video-fluoroscopy will be taken during three weeks before the commencement of the training to obtain baseline assessment. Measurements of maximum inspiratory pressure and maximum expiratory pressure will be performed over 3 testing sessions with a week apart to assess the test-retest reliability. Spirometry, maximum inspiratory pressure and maximum expiratory pressure will be repeated at 1 and 2 months after training. Peak expiratory flow (reflex and voluntary cough) and video-fluorographic analysis of swallowing will be repeated at 2 months after training. All measurements will be performed at the same place and same time of the day. To minimize possible acute effects of the training session on the measures, 1 or 2 days will be inserted between the training session and the measurements.

Same measurement protocol used in study 1 for maximum inspiratory pressure, maximum expiratory pressure, spirometry, voluntary and reflex cough and video-fluorographic measurement of swallow will be applied in study 2.

Statistical Analysis

Descriptive data will be given as mean, standard deviation and range. To compare physical and physiological characteristics and baseline measurements between study groups, a one-way ANOVA will be performed. To assess the test-retest reliability of maximum inspiratory pressure and maximum expiratory pressure measurements, an interclass correlation coefficient (ICC) and the coefficient of variation (CV) will be calculated for each study group. Normality assumption will be tested using the Shapiro-Wilk test. Changes in pulmonary function parameters due to group and or time effect will be tested using repeated measures ANOVA. Changes in the penetration/aspiration scale will be analysed using Friedman´s analysis of variance test and the Wilcoxon signed rank sum test. Statistical significance will be set at $P \leq 0.05$. Statistical analyses will be performed using STATA statistical software version 9.1

WORK PLAN

In this proposed research the work plan is to perform and complete each study in one year.

The tasks for year 1 are:

1. Purchasing equipment that includes the pneumotachograph and respiratory muscle trainers devices
2. Patients recruitment for study 1
3. Data collection and analysis for study 1
4. Writing two manuscripts from data collection of study 1
5. Mansucripts submission

The tasks for year 2 are:

1. Purchasing equipment that includes respiratory muscle trainers devices, manual resucitator bags and facemaks
2. Patients recruitment for study 2
3. Data collection and analysis for study 2
4. Writing a third manuscript
5. Mansucripts submission
6. Results presentation in an International Conference (midyear 2019)

		Year 1												Year 2											
task	**Goal/month**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	Equipment purchase1	x	x	x																					
2	Patients recruitment study 1			x	x	x																			
3	Data collection study 1					x	x	x	x																
3	Data analysis study 1								x	x															
4	Writing manuscript 1									x	x	x													
4	Writing manuscript 2										x	x	x												
5	Manuscripts submission												x												
1	Equipment purchase2												x	x	x										
2	Patients recruitment study 2													x	x	x									
3	Data collection study 2															x	x	x	x						
3	Data analysis study 2																		x	x	x				
4	Writing manuscript 3																				x	x	x	x	
5	Manuscripts submission																							x	
6	International conference																								

Work in progress
The author of this proposed research has experience using respiratory muscle and limb strength training in patients with neurodegenerative disorders. The author also has experience performing most of the pulmonary function measurements described in this proposal. This research project intends to be attractive for Parkinson´s disease patients because it may have an impact on coughing and swallowing that are sensitive functions for PD patients. The author of this proposal has experience promoting and contacting patients for research purposes. Currently we are recruiting participants with Parkinson´s disease for a study which purpose is to treat swallowing difficulties and prevent aspiration pneumonia events. This current study will help us to facilitate the access to the community of patients with PD and to consolidate networking among neurologist, radiologist, physical therapists and other health care professionals that will participate in this proposed research project.

Available Resources

Exercise Physiology Laboratory
The exercise physiology laboratory of the School of Kinesiology at Pontificia Universidad Catolica de Chile provides spirometers (MicroLab^{TM}), pressure manometers (Micro RPM, Micro Medical-Care Fusion, UK. POWER breathe K5) and peak cough flow meters (Mini-Wright, Clement-Clarke) to run the assessments. This laboratory also has comfortable space for patients to perform the assessments.

X ray department
We also count with the collaboration of the X ray department of the Hospital Clinico at UC-Christus Health network, to perform video-fluoroscopy.

Respiratory diseases Laboratory
The respiratory diseases laboratory at Hospital Clinico UC also has the facilities and the equipments to perform spirometry and respiratory muscle strength measurements.