Impact of Lung Boost Exerciser on pulmonary functions in iron deficiency anaemia

Sara Rabie Ahmed
Department of Physical Therapy for Cardiovascular / Respiratory Disorders and Geriatrics Faculty of Physical Therapy, Misr University for Science and Technology, Egypt.
Email: sara.rabee@must.edu.eg

Nagwa Mohamed Hamed Badr
Department of Physical Therapy for Cardiovascular / Respiratory Disorders and Geriatrics, Faculty of Physical Therapy, Cairo University, Egypt.

Abla Mohamed Hamed
Department of Physical Therapy for Cardiovascular / Respiratory Disorders and Geriatrics Faculty of Physical Therapy, Misr University for Science and Technology, Egypt.

Fatma Abdelkader Attia
Department of Internal Medicine, Faculty of Medicine for Girls, AlAzhar University, Egypt.

Heba Ahmed Ali Abdeen
Department of Physical Therapy for Cardiovascular / Respiratory Disorders and Geriatrics, Faculty of Physical Therapy, Cairo University, Egypt.

Abstract---This prospective study's objective was to determine the impact of lung Boost exerciser on pulmonary functions in iron deficiency anaemia. Forty patients (18 males and 22 females) with iron deficiency anaemia were recruited from haematology department in AL-Kasr Al Ainy hospital where the study was conducted. Participants were aged between 20 to 35 years old. They were assigned randomly into two groups; study group(A) which were twenty patients received lung boost exerciser program for thirty minutes, three times/week in addition to medical treatment and twenty patients in control group(B) received medical treatment only. The exercise lasted from 5-20 minutes including 15 deep breaths, and 15-second rest in between. The total treatment period for both groups was twelve weeks. Pulmonary Functions which included Peak Expiratory Flow (PEF), Forced Vital Capacity (FVC), Forced Expiratory Volume in the First Second (FEV1), and the ratio of FEV1 to FVC (FEV1/FVC) were evaluated for all
patients in both groups at the beginning of the study and after 12 weeks by using spirometer. There were significant (P<0.05) increases in FVC, FEV1, and PEF at post-treatment compared to pre-treatment within the study group (P=0.0001, P=0.0001, and P=0.0001, respectively), according to the statistical analysis by multiple pairwise comparisons (pre-treatment vs. post-treatment) within each group. However, the increase in the control group was non-significant (P>0.05) (P=0.527, P=0.552, and P=0.375, respectively) at post-treatment when comparing to pre-treatment. Study group improved higher FVC, FEV1, FEV1/FVC ratio, and PEF (20.44, 23.56, 2.58, and 27.88%, respectively) than control group (2.56, 1.93, 0.62, and 4.47%, respectively). It was revealed that lung boost exerciser is efficient in increasing pulmonary functioning in individuals with iron deficiency anaemia.

**Keywords**—Lung Boost Exerciser, pulmonary functions, iron deficiency anaemia.

1. **Introduction**

Anaemia occurs when there are not enough red blood cells (RBCs) in the blood or the amount of haemoglobin in the blood cells is lower than usual. The role of red blood cells is to deliver oxygen from the lungs to the rest of the body. The functional unit is a complex protein structure called the haemoglobin molecule that is located inside RBCs. Even though RBCs are generated in the bone marrow, they are affected by many factors. Iron, for example, is a critical component of haemoglobin; erythropoietin, a protein released by the kidneys, encourages RBC synthesis in the bone marrow. (Abbaspour et al., 2014).

Anaemia is divided into numerous types and classes. Aplastic anaemia, iron deficiency anaemia, thalassemia, genetic disorders in haemoglobin maturation, haemoglobinopathies, sickle cell anaemia, thalassaemia and other red cell abnormalities can all result in anaemia, as well as physical red cell loss (haemolytic anaemias) (Mukherjee and Ghosh, 2012).

Iron deficiency anaemia (IDA) is characterized by a low concentration of circulating RBCs and a low quantity of haemoglobin in the blood cells. This results in a reduction in oxygen transport to tissues, iron storage, and iron dependent oxidative enzymes. (Coad and Conlon 2011). Enzymatic reactions, transport of oxygen, synthesis of DNA, and production of mitochondrial energy are just a few of the cellular functions that require iron. (Lopez et al.2016).

The signs and symptoms of iron deficiency anaemia are varied. Breathing difficulties, fatigue, palpitations, arrhythmias, and angina may result from low blood oxygen levels. Hypoxemia can result in a compensating reduction in intestinal blood circulation, which can cause gastrointestinal problems, nausea, losing weight, and abdominal discomfort. Central hypoxia can produce headaches, dizziness, and tiredness, as well as cognitive impairment. Multiple
researches have shown that after anaemia is treated, cognitive functions improve. (Cappellini et al., 2017).

Anaemia, a common occurrence, interferes with the body’s normal metabolism due to anaemic hypoxia, which can have a variety of systemic effects, one of them is the respiratory system. (Gupta and Dixit, 2011). Some spirometric parameters may be affected by microcytic anaemia (mostly FVC and to a lesser degree FEV1). Even though the differences are minor, adjusting them can assist minimise overestimation of lung problems, especially in borderline instances. (Imanizade and Danesh, 2019).

Lung Boost is a device used to strengthen the respiratory muscles. Numerous studies have shown that using a Respiratory Muscle Training (RMT) device in conjunction with a fitness routine such as walking results in increased respiratory muscle strength and endurance, as well as other benefits such as longer walking distance, reduced shortness of breath, improved quality of life, and a general sense of well-being. (Amisha and Vishnu, 2021). Inspiratory Muscle Training (IMT) improves ventilatory function, respiratory strength, endurance and functional ability and also help to reduce dyspnea (Neves et al., 2014).

2. Methods

2.1. Participants

Forty patients from both sexes (18 male and 22 female) with iron deficiency anaemia were screened for eligibility to join the research. Patients were selected from the hematology department in AL-Kasr Al Ainy hospital, where the study was conducted after they had fulfilled the inclusion criteria of the study. They were aged between 20 to 35 years old. They were diagnosed and examined by a physician. They were diagnosed as iron deficiency anaemia from 4 to 5 years. They received regular medical treatment. The human research ethical committee at Faculty of Physical Therapy, Cairo University approved the study. The practical part in the study lasted from 15 January 2020 to 30 October 2021.

However, Patients were ruled out if they had respiratory tract infection symptoms within last two weeks, any previous cervical or thoracic surgery, clinical signs of a severe cardiac event, severe psychiatric or cognitive impairment and neurological disorders affecting respiratory muscles or any muscular dystrophies.

Design of the study

This research was planned as a parallel, randomized, single-blinded study. With active treatment groups. Before participation in the study, patients received a full explanation about the background, objectives, and advantages of the study. All participants fully understood the purpose and procedures of the research and they were given informed consent form to sign before the study procedures, and it was clearly stated that they are free to refuse or decline from participation in the research at any stage, and their personal information is to be kept confidential. They were divided to two groups as follows: twenty patients in study group (A) and twenty patients in control group (B). An independent research assistant used randomly generated numbers from a computer to randomize the data.
2.2. Instruments

Pulmonary functions (FEV1, FVC, FVC/FEV1 ratio and PEF) for both groups were assessed using the electronic spirometer (Contec SP 10W spirometer (Model - Schiller AG, CH6304)). For group (A) Lung Boost Respiratory Trainer was used as a strengthener of respiratory muscles which results in improving of lung capacity and subsequently improving of ventilation for group (A). Strength and endurance modes are its two training modes. Training for lung breath time duration is done in the endurance mode. This phase is attained by inhaling or exhaling as long as possible. The number of the highlighted ball on the screen represented the lung effort level. Strength mode designed to improve lung strength. It acts through inhale or exhale as possible. (Lee, 2020).

2.3. Procedures

A full explanation of the study details was offered to all participated patients before starting the study. They were clinically examined by the physician to be diagnosed as iron deficiency anaemia and excluded any associated conditions and then all patients followed the same evaluative procedures. The participants were instructed to report any side effect during the applications. A written consent was obtained from each patient.

2.3.1. Assessment

Spirometer was used to measure Pulmonary Functions which include Peak Expiratory Flow (PEF), Forced Vital Capacity (FVC), Forced Expiratory Volume in the First Second (FEV1), and the ratio of FEV1 to FVC (FEV1/FVC) at the beginning of the study and after 12 weeks.

- **Forced Vital Capacity (FVC):** is maximum quantity of air that can be expelled following full inhalation.
- **Forced Expiratory Volume in 1 second (FEV1):** is the volume of air that can be forcibly expelled at the first second of FVC after full inspiration (Perez, 2012).
- **FVC and FEV1** were measured by having the patient inhale deeply and as fully as possible then exhaling quickly and forcefully until no more air could be expelled from the lungs (Rudraraju et al., 2020).
- **FEV1/FVC ratio:** is the FEV1 to FVC ratio (Philadelphia and Saunders 2010).
- **Peak Expiratory Flow Rate (PEF):** is the highest flow (or velocity) reached following a maximally forced expiration, measured in litres per minute
- **This procedure was carried out at least three times** for each parameter before selecting the most accurate result. After the PFT data had been normalised for age, gender, and height as a percentage, the estimated FEV1 and FVC values were determined. After two or three more regular and complete breaths, the measurement was completed (Minnella, et al., 2017).
### 2.3.2. Intervention

**Group A (Study Group):** included Twenty patients (8 male and 12 female) with iron deficiency anaemia who received lung boost exerciser program for thirty minutes, three times/week in addition to medical treatment for twelve weeks. The procedure and the aim of the device were explained and demonstrated to all participants. The participants were trained on the device prior to the exercise. The participant looked at the respiratory exercise device with a mouthpiece over his/her mouth and Inhalation was performed (Lee, 2020). The participants received the strength mode (high intensity interval training) by pressing on the strength mode button then choose the difficulty level by pressing on difficulty level button. The training starts with the easiest difficulty level 1 (default) and then put the mouth piece inside his/her mouth and inhale through the mouth piece as much as possible then the effort was indicated by the number of balls appeared on the right side of the screen which gave the participant excellent feedback for his actual effort and improvement. If the first level was easy for the participant, he/she was advanced for the following difficult level at a time until the desired difficulty level was achieved. The exercise lasted from 5-20 minutes including 15 deep breaths, and 15-second rest in between (Ahmed et al., 2021).

**Group B (Control Group):** included Twenty patients (10 male and 10 female), with iron deficiency anaemia who received medical treatment only with total treatment period twelve weeks. They considered as a control group. The same whole initial assessment procedure was repeated again after the 12 weeks of treatment for all patients in both groups.

### 2.4. Statistical analysis

The homogeneity of variance test and the normality assumption test were used to screen the data. Following the removal of outliers identified using box and whiskers plots, the Shapiro-Wilk test was used to determine the normality of the data, which revealed that the data was normally distributed (P>0.05). There was also no significant difference, as determined by Levene’s test for homogeneity of variance (P>0.05). The data are therefore regularly distributed, and parametric analysis is performed. The SPSS Package programmed for Windows, version 25(SPSS, Inc., Chicago, IL), was used to conduct the statistical analysis. Data on height, age, weight, BMI, FEV1, FVC, FEV1/FVC ratio, and PEF are expressed quantitatively as mean and standard deviation, and data on gender are expressed qualitatively as number and percentage. To compare between 2 groups by independent t-test for age, weight, height, and BMI variables and chi-square test for gender variable. A multivariate analysis of variance (MANOVA) was conducted to compare the evaluated main variables of interest (FVC, FEV1, FEV1/FVC, and PEF) at varied tested groups and during various measurement intervals. between the iron group and the control iron group. Bonferroni adjustment test was conducted to compare the tested variables pairwise within and across groups where the F value from the MANOVA test was significant. At the level of probability, all statistical analyses were significant (P 0.05).
3. Results

In the present study, total of 40 patients of both genders (18 male and 22 female) were assigned randomly into 2 groups (20 patients/group). No significant differences (P>0.05) in demographic data for age (P=0.738), weight (P=0.238), height (P=0.722), BMI (P=0.386), and gender (P=0.525) between study group and control group (Table 1).

<table>
<thead>
<tr>
<th>Items</th>
<th>Groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group (n=20)</td>
<td>Control group (n=20)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>23.79 ±1.93</td>
<td>24.05 ±2.74</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.30 ±4.65</td>
<td>54.75 ±3.41</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.63 ±4.75</td>
<td>159.09 ±4.89</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>22.13 ±2.33</td>
<td>21.63 ±1.20</td>
</tr>
<tr>
<td>Gender (males: females)</td>
<td>8 (40%):12 (60%)</td>
<td>10 (50%):10 (50%)</td>
</tr>
</tbody>
</table>

Quantitative data (age and BMI) are compared using the t-independent test and expressed as mean± standard deviation. The chi-square test is used to compare qualitative data (gender, M: male and F: female) that are expressed as numbers (percentages) P-value: probability value; NS: non-significant

There were significantly (P<0.05) increased FVC, FEV1, and PEF at post-treatment compared to pre-treatment within study group (P=0.0001, P=0.0001, and P=0.0001, respectively) according to the statistical analysis by multiple pairwise comparisons (pre-treatment vs. post-treatment) within each group (Table 1). However, a non-significant (P>0.05) rise was shown in the control group at post-treatment compared to pre-treatment (P=0.527, P=0.552, and P=0.375, respectively). Moreover, no significant differences (P>0.05) in FEV1/FVC ratio within study group (P=0.551) and control group (P=0.459) between pre-and post-treatment. The study group improved higher FVC, FEV1, FEV1/FVC ratio, and PEF (20.44, 23.56, 2.58, and 27.88%, respectively) than control group (2.56, 1.93, 0.62, and 4.47%, respectively).

There were no significant differences (P>0.05) between the study group and control group at pre-treatment for FVC (P=0.734), FEV1 (P=0.800), and PEF (P=0.571), according to the statistical analysis of multiple pairwise comparisons (study group vs. control group) for outcomes variables (Table 2). However, there were significant differences (P<0.05) between group A and group B in FVC (P=0.0001), FEV1 (P=0.0001), and PEF (P=0.0001). Both pre-treatment (P=0.732) and post-treatment (P=0.096) FEV1/FVC ratios showed no significant differences (P>0.05). These significant and non-significant increases in FVC, FEV1, FEV1/FVC ratio, and PEF at post-treatment favor of study group (75.12 ±6.28, 79.35 ±5.26, 105.63 ±5.98, and 74.99 ±5.63, respectively) than control group (64.87 ±7.24, 64.92 ±7.00,100.08 ±6.12, and 63.06 ±11.75, respectively).
Table 2: Mixed MANOVA for outcomes variable comparisons within and between groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Items</th>
<th>Study group (n=20)</th>
<th>Control group (n=20)</th>
<th>Change</th>
<th>P-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>Pre-treatment</td>
<td>62.37 ±7.34</td>
<td>63.25 ±10.74</td>
<td>0.88</td>
<td>0.734</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>75.12 ±6.28</td>
<td>64.87 ±7.24</td>
<td>10.25</td>
<td>0.0001*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>change</td>
<td>12.75</td>
<td>1.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improvement %</td>
<td>20.44%</td>
<td>2.56%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.0001*</td>
<td>0.527</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1</td>
<td>Pre-treatment</td>
<td>64.22 ±5.80</td>
<td>63.69 ±7.59</td>
<td>0.52</td>
<td>0.800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>79.35 ±5.26</td>
<td>64.92 ±7.00</td>
<td>14.43</td>
<td>0.0001*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>change</td>
<td>15.13</td>
<td>1.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improvement %</td>
<td>23.56%</td>
<td>1.93%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.0001*</td>
<td>0.552</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>Pre-treatment</td>
<td>102.97 ±11.92</td>
<td>100.70 ±15.32</td>
<td>2.27</td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>105.63 ±5.98</td>
<td>100.08 ±6.12</td>
<td>5.55</td>
<td>0.096</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improvement %</td>
<td>2.66</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>change</td>
<td>2.58%</td>
<td>0.62%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.551</td>
<td>0.459</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF</td>
<td>Pre-treatment</td>
<td>58.64 ±9.53</td>
<td>60.36 ±10.21</td>
<td>1.72</td>
<td>0.571</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>74.99 ±5.63</td>
<td>63.06 ±11.75</td>
<td>11.93</td>
<td>0.0001*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>change</td>
<td>16.35</td>
<td>2.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improvement %</td>
<td>27.88%</td>
<td>4.47%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.0001*</td>
<td>0.375</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (SD). P-value: probability value.

The mean values of FVC, FEV1, FEV1/FVC ratio, and PEF improvement percentage in study group were 20.44, 23.56, 2.58, and 27.88%, respectively, in control group 2.56, 1.93, 0.62, and 4.47%, respectively. According to the statistical analysis, there was a considerable increase in FVC (P=0.0001; P<0.05), FEV1 (P=0.0001; P<0.05), FEV1/FVC ratio (P=0.005; P<0.05) and PEF (P=0.0001; P<0.05) improvement percentage in study group when compared to control group (Table 3).
Table 3: Comparison of the improvement percentage mean of the outcome variables between the study group and the control group

<table>
<thead>
<tr>
<th>Items</th>
<th>Study group (n=20)</th>
<th>Control group (n=20)</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>20.44%</td>
<td>2.56%</td>
<td>0.0001*</td>
</tr>
<tr>
<td>FEV1</td>
<td>23.56%</td>
<td>1.93%</td>
<td>0.0001*</td>
</tr>
<tr>
<td>FEV1/FVC ratio</td>
<td>2.58%</td>
<td>0.62%</td>
<td>0.005*</td>
</tr>
<tr>
<td>PEF</td>
<td>27.88%</td>
<td>4.47%</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (SD)  

P-value:  
* Significant (P<0.05)

4. Discussion

Anemia reduces the body's normal functions, including the respiratory system. Oxygen is required for each cell and whole biological system for optimum functioning. It is essential for a variety of metabolic pathways involved in dietary nutrient catabolism. Protein synthesis and a variety of other substances are produced as a result of proper cell functioning, providing strength to all muscles. (Jain and Agarwal, 2014).

The results of this study revealed that within the study group (group A), there was a substantial (P<0.05) increase in FVC, FEV1, and PEF at post-treatment compared to pre-treatment (P=0.0001, P=0.0001, and P=0.0001, respectively). However, a non-significant (P>0.05) rise was shown in the control group (group B) (P=0.527, P=0.552, and P=0.375, respectively) post-treatment compared to pre-treatment. There were no significant variations (P>0.05) in the FEV1/FVC ratio between the two groups at either the pre-treatment (P=0.732) or post-treatment (P=0.096). The study group improved higher FVC, FEV1, FEV1/FVC ratio, and PEF (20.44, 23.56, 2.58, and 27.88%, respectively) than control iron group (2.56, 1.93, 0.62, and 4.47%, respectively). These significant and non-significant increases in FVC, FEV1, FEV1/FVC ratio, and PEF at post-treatment favor of study group (75.12 ±6.28, 79.35 ±5.26, 105.63 ±5.98, and 74.99 ±5.63, respectively) than control group (64.87 ±7.24, 64.92 ±7.00, 100.08 ±6.12, and 63.06 ±11.75, respectively).

The results of the current study regarding to increasing pulmonary functions in study group agreed with the results achieved by Alotaibi and Waked (2015) which stated that in patients with sickle cell anaemia, incentive spirometry proved beneficial in improving pulmonary function and thereby reducing pulmonary problems.

Adeniyi AF and Saminu KS., 2011 mentioned that in sickle cell anaemia patients, a three-day incentive spirometry using a locally apparatus resulted in a significant improvement in PEFR from the third to the sixth week. Exercise that improves PEFR should be done on a regular basis in order to improve a patient’s pulmonary status and lung function.

In agreement with our results Camcioglu B et al., 2015 reported that after using an inspiratory muscle trainer for 12 weeks daily for 30 minutes, 7 days/week, there was an improvement in respiratory muscle strength, objective and
subjective manifestations. The presenting patients presented with obstructive and restrictive pulmonary function patterns, obstruction in small airways, and decreased pulmonary diffusion capacity, which were similar to the pulmonary function abnormality pattern.

Regarding significance improvement in FVC and FEV1 in study group post treatment compared to control group, our results agreed with the results of the study conducted by Instar S. Waked (2017) which showed significant increase in pulmonary functions (FVC, FEV1, diffusion capacity (DLCO)) in experimental group when compared with control group in fifty-four patients with homogenous sickle cell anaemia after applying inspiratory training using incentive spirometry for ten minutes, three times per week for eight weeks.

Our study's findings are in conflict with those of a study conducted by Galvao et al. (2020), which found no significant difference between pre- and post-treatment in lung functions in two groups of sickle cell disease patients using inspiratory muscle trainer device (POWER breathe) for 18 weeks. They were divided into true load group who performed the inspiratory muscle training with a true load and the sham load group who performed with no true load. Patients from both groups were directed to use the inspiratory muscle trainer every day for 10 minutes, with a 1-minute rest after every 5 minutes, for the duration of 18-week training period. During the 10 minutes of daily exercise, between 13 and 15 breaths per minute were repeated.

Anemia can cause mild to severe weakness in the respiratory muscles, as well as decreased cardiopulmonary effectiveness and fatigability. Oxygen is given to tissues via blood in two forms: dissolved and bonded with haemoglobin. Reduced oxygen availability to tissues impairs cell activity, which in turn impacts organ systems, including respiratory muscles, worsening oxygen uptake by lungs from the atmosphere. Reduced pulmonary functions are caused by weakness of respiratory muscles, especially the diaphragm. (Gupta L and Dixit R., 2011).

Inspiratory muscle training, on the other hand, increases not only respiratory muscle strength but also peak VO2, exercise capacity, quality of life, strength of the peripheral muscles and oxygen uptake efficiency. (Gosselink et al., 2011). From this study's findings as well as those of earlier studies, it was concluded that lung boost exerciser was effective in improving pulmonary functions so decreasing pulmonary problems in patients with iron deficiency anaemia.

**Author Contributions**
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

**Conflicts Of Interest**
The authors declare no conflict of interest.

**Funding**
This research received no external funding.
References


