Physiological Effects of Concurrent Training in Elderly Men

Authors
E. L. Cadore¹, R. S. Pinto¹, F. L. R. Lhullier², C. S. Correa¹, C. L. Alberton¹, S. S. Pinto¹, A. P. V. Almeida¹, M. P. Tartaruga¹, E. M. Silva¹, L. F. M. Kruel¹

Affiliations
¹Federal University of Rio Grande do Sul, Physical Education School, Porto Alegre, Brazil
²Catholic Pontifical University of Rio Grande do Sul, Department of Clinical Analysis, Porto Alegre, Brazil

Key words
- physical training
- neural adaptations
- serum hormones
- strength development

Abstract
The aim of the present study was to investigate the effects of concurrent strength and endurance training on neuromuscular and hormonal parameters in elderly men. 23 healthy men (65 ± 4 years) were randomly assigned to 1 of 3 groups: concurrent (CG, n = 8), strength (SG, n = 8) or endurance group (EG, n = 7). The programs consisted of strength training, endurance training on a cycle ergometer or a combination of both in the same session 3 times per week over a duration of 12 weeks. Subjects were evaluated on parameters related to muscle strength, muscle activation and serum hormones. There were significant increases in lower-body strength in all groups (P<0.05), with higher increases in SG (67%) than CG (41%) and both were higher than EG (25%) (P<0.01). Only SG and CG increased upper-body strength (P<0.01), with no significant difference between the 2 groups. Furthermore, there were significant decreases in free testosterone in EG after training. Significant increases in isometric strength and maximal muscle activation (P<0.05) as well as decreases in the submaximal muscle activation to the same load, were only seen in SG (P<0.05). The present results suggest that the interference effect observed due to concurrent strength and endurance training could be related to impairment of neural adaptations.

Introduction
Biological aging is associated with a decline in the capacity to develop force and power [24], reduced cardiorespiratory fitness [19] and consequently decreased functional capacity, especially after the decade of life between 60 and 70 [10,12]. To counteract this, several studies have shown that strength training improves both strength and power during aging [13–15] and that endurance training also enhances aerobic fitness in this population [21,23]. In view of this, the prescription of both endurance and strength training is fundamental to improve functional capacity in elderly populations [2].

The combination of strength and endurance training (i.e., concurrent training) has been widely investigated [8,23,31,37]. These studies have shown that combining endurance and strength training, specifically when high volumes, intensity and/or frequencies are employed, may inversely affect the gains observed during strength and power training (i.e., interference effect) [7,25,36]. However, few studies have investigated the interference effects in elderly subjects. Wood et al. [40], investigating elderly men, demonstrated that 12 weeks of concurrent training resulted in similar strength gains to those observed with strength training alone. However, the authors used 50% lower volume of strength training in the concurrent training group [40]. Similarly, Izquierdo et al. [20] observed no differences between strength (twice weekly) and concurrent training (strength exercises on 1 day, cycle ergometer on the other) in the strength gains. In these 2 studies involving elderly subjects, the volume of training performed was lower in the concurrent training groups [20,40]. Thus, it is not known whether interference would have occurred if these 2 studies had used similar volumes for strength training in the concurrent groups as they used in the strength training alone groups. However, it is possible that even by performing a higher volume of concurrent training, the large trainability of untrained elderly subjects may lead to similar strength enhancements induced by concurrent and strength training with no presence of interference effect in this population [23].

Several mechanisms have been suggested as being responsible for the interference of endurance exercise on strength gains resulting from
resistance training. These include negative effects on neural adaptations [15], low glycogen content resulting in chronic catabolic state [4, 25]; and, interference on the hypertrophy of type I fibers [3, 35]. However, there is a lack of evidence of interference on the neural component of strength development when evaluated by electromyography (EMG) measurements [15, 31]. Hákkinen et al. [15] have shown that only the strength group enhanced fast muscular activation (500 ms) after training compared with concurrent group. In another study, McCarthy et al. [31] did not find any differences between strength and concurrent groups in the magnitude of neural adaptations (maximal EMG amplitude) after training. Nevertheless, no interference effect on maximal strength was observed in these studies [5, 31], and a question that arises is: could the neural component explain the interference effect, in the presence of this phenomenon? The chronic catabolic state has been investigated using resting serum hormonal concentrations (i.e., testosterone, cortisol) such as anabolism/catabolism markers during the training period. In fact, studies investigating both strength and concurrent training modalities have demonstrated that when high training volume is performed, different hormonal modifications may ensue [5, 25]. Besides this possibility, the influence of the reduction in testosterone on the strength decline during aging [12] provides the rationale for conducting research in this area. Likewise, these endocrine adjustments have yet to be investigated in older populations performing concurrent training. Therefore, the aim of the present study was to investigate the neuromuscular and hormonal adaptations that occur in response to strength, endurance and concurrent training in elderly subjects. Our hypothesis was that, due to the large trainability of older subjects, no differences between strength and concurrent training would be found. However, if the interference effect was observed, we speculated that neural and endocrine mechanisms could help to explain this possible effect.

Methods

Experimental design

In order to investigate the adaptations to strength, endurance and concurrent training, subjects were evaluated using variables related to maximal strength, muscular activation and serum hormonal concentrations. The investigations of electromiographic signal (EMG) and hormonal parameters would provide insights about possible mechanisms involved with training adaptations or even an interference effect. The total duration of the present study was 16 weeks, in which the subjects were tested at -4 and 0 weeks before (control period) and 12 weeks after each specific training program. Thus, all the tests were performed 3 times during the investigation and the first and second pre training measures (control period) were used to determine the stability and reliability of the variables. Each subject performed the tests at the same time of day throughout the period of the study and the different tests were conducted on different days to avoid fatigue. At each point of evaluation (control, pre and post training), subjects completed all the tests in 1 week.

Subjects

29 healthy elderly men (Mean±SD: 65±5 years; age range: 61–70 years), who had not been engaged in any regular and systematic training program in the previous 12 months, volunteered for the study after completing an ethical consent form. The subjects were randomly selected and placed into 3 groups: strength training (SG, n=10); endurance training (EG, n=9); and, concurrent training (CG, n=10). 3 participants dropped out after the control period and another 3 dropped out during the training period. 4 of them due to professional problems and the other 2 moved to another city. At the end of the study, the number of subjects in each group was: SG=8; EG=7; and, CG=8. The study was conducted according to the ethical standards of the International Journal of Sports Medicine described by Harris and Atkinson [17], and was approved by Ethics Committee of Federal University of Rio Grande do Sul, Brazil. Exclusion criteria included any history of neuromuscular, metabolic, hormonal and cardiovascular diseases. Subjects were not taking any medication with influence on hormonal and neuromuscular metabolism and were advised to maintain their normal dietary intake throughout the study. Medical evaluations were performed using clinical anamnesis and effort electrocardiograph test (ECG), to ensure subject suitability for the testing procedure. The physical characteristics of subjects are shown in the Table 1.

Body composition

Body mass and height were measured using an Asimed analog scale (resolution of 0.1 kg) and an Asimed stadiometer (resolution of 1 mm), respectively. Body composition was assessed using the skinfold technique. The same technician obtained all anthropometric measurements on the right side of the subject’s body. Skinfold thickness was obtained with a Cescorf skinfold caliper. A 7-site skinfold equation was used to estimate body density [22] and body fat was subsequently calculated using the Siri equation [18].

Table 1  Physical characteristics before and after training. Mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>Concurrent training</th>
<th>Strength training</th>
<th>Endurance training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG, n=8</td>
<td>SG, n=8</td>
<td>EG, n=7</td>
</tr>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
</tr>
<tr>
<td>age (years)</td>
<td>66.8±4.8</td>
<td>67.6±5.3</td>
<td>64.0±3.5</td>
</tr>
<tr>
<td>body mass (kg)</td>
<td>85.3±11.9</td>
<td>89.7±10.7</td>
<td>80.8±12.2</td>
</tr>
<tr>
<td>height (cm)</td>
<td>176.6±4.8</td>
<td>176.6±4.8</td>
<td>173.4±6.2</td>
</tr>
<tr>
<td>% fat mass</td>
<td>26.2±3.9</td>
<td>28.8±2.9</td>
<td>27.3±2.7</td>
</tr>
<tr>
<td>fat mass (kg)</td>
<td>22.7±6.3</td>
<td>25.1±5.7</td>
<td>22.2±5.0</td>
</tr>
<tr>
<td>VO2peak (ml·kg⁻¹·min⁻¹)</td>
<td>27.0±3.7</td>
<td>32.9±2.7*</td>
<td>27.3±4.3</td>
</tr>
</tbody>
</table>

* Significant difference from pre training values (P<0.05)
Maximal dynamic strength

Maximal strength was assessed using the 1-repetition maximum test (1-RM) on the bench press and bilateral knee extension. 1 week prior to the test day, subjects were familiarized with all procedures. On the test day, the subjects warmed up for 5 min on a cycle ergometer, stretched all major muscle groups, and performed specific movements for the exercise test. Each subject's maximal load was determined with no more than 5 attempts with a 4-min recovery between attempts. Performance time for each contraction (concentric and eccentric) was 2 s, controlled by an electronic metronome (Quartz, CA, USA). The test-retest reliability coefficient (ICC) was 0.99 for both exercises.

Maximal isometric strength

In order to obtain the maximal isometric strength, the subjects warmed up for 5 min on a cycle ergometer and were then positioned on knee extension exercise machine (Taurus, Porto Alegre, Brazil), fitted with a load cell coupled to the cable that displaced the load. The load cell was connected to an A/D converter (Miotec, Porto Alegre, Brazil), which made it possible to quantitate the traction exerted when each subject executed the knee extension at the determined angle. The subjects were positioned seated with the hip at a 110° angle, strapped to the apparatus. The inter-electrodes distance was 20 mm, corresponding to the angle of over 70 rpm. All the incremental tests were conducted with the same angle, with 5-min rest between trials. The apparatus and the collection and analysis procedures were the same used to determine the maximal EMG signal. After the training period, the submaximal muscular activation was determined for the same absolute loads used in the pre-training evaluation. The submaximal RMS values were normalized using the maximum RMS values obtained during the MVC in each muscle. The test-retest reliability coefficient (ICC values) of the EMG measurements was over 0.85.

Blood collection and analysis

Blood was obtained from the subjects between 8:00 and 8:30 a.m., after 8 h of sleep, 12 h fasting and 2 days with no exercise. The time of blood collection was chosen due to its use in many studies conducted with these procedures for the control of the circadian hormonal range [6,12]. Subjects sat in a slightly reclined position for 15 min after which 10 ml of blood was drawn from an antecubital vein. After collection, the blood was heated up for 5 min on a cycle ergometer and was then positioned at the isometric neuromuscular economy. Thus, subjects randomly warmed up for 5 min on a cycle ergometer and were then positioned on a brief test (1-RM) on the bench press and bilateral knee extension.
The strength training program was designed to improve muscular endurance in the first 4 weeks and subsequently to stimulate muscular hypertrophy and maximal strength gains. Before the start of the strength training, subjects completed 2 familiarization sessions to practice the exercises they would further perform during the training period. The individuals in the SG performed 9 exercises (inclined leg-press, knee extension, leg curl, bench press, lat pull down, seated row, triceps curl, biceps curl and abdominal exercises) 3 times a week on non-consecutive days. In each session, subjects performed specific muscle stretching and a specific warm up, with one set of 25 repetitions with very light loads for the upper and lower body. During weeks 1–7, subjects performed 2 sets of 18–20 repetitions maximum (RM) in week 1 (i.e., the heaviest possible weight was used for 1–7 repetitions), progressing to 12–14 RM in week 5. In weeks 8–12, subjects performed 3 sets of 12–14 RM (week 8), advancing to 6–8 RM (week 11). During the training program, although the exact percent of 1 RM in each set was not controlled, all the sets were performed until failure [25,36]. In each set the workload was adjusted when the repetitions performed were either above or below the repetitions established. The recovery time between sets was 90–120 s. The SG training sessions lasted approximately 40 min. The whole strength training periodization is shown in Table 2. All the training sessions were carefully supervised by at least 2 experienced personal trainers.

Endurance training
Participants in the EG trained 3 times a week, on non-consecutive days, using a cycle ergometer, at the intensity relative to the heart rate (HRVT) corresponding to the second ventilatory threshold (VT2). In each session, subjects performed a warm up lasting 5 min at comfortable cadence. During the first 2 weeks, subjects cycled for 20 min at 80% of HRVT, progressing to six 4-min bouts at 100% of HRVT (weeks 11–12), with 1 min of active recovery between bouts. The VT2, used as a parameter to prescribe the intensity of endurance training, corresponded to 79.6±5% of the VO2peak. The whole endurance training periodization is shown in Table 2.

Concurrent training
Participants in the CG performed the combined volume of both the SG and EG training. During all sessions, strength exercises were preceded by endurance exercise. This order of exercise was chosen to investigate the effects of aerobic training preceding strength training due to the possible fatigue effects of aerobic training, especially when performed on a cycle ergometer [28]. The CG training sessions lasted approximately 70 min.

Statistical analysis
The SPSS statistical software package was used to analyze all data. Results are reported as mean±SD. Statistical comparisons in the control period (from week 0 to 6 weeks) were performed using Student’s paired t-tests. The training-related effects were assessed using a 2-way Analysis of Variance (ANOVA) with repeated measures (group x time). When a significant F value was achieved, Bonferroni post-hoc procedures were used to locate the pairwise differences. Selected relative changes between groups were compared via 1-way ANOVA. Significance was accepted when p<0.05.

Results
All subjects performed at least 85% of training with no difference between groups in the number of sessions performed (CG and SG: 32 of 36 and EG 33 of 36 sessions). The results are shown as Mean±SD. There were no differences between the groups in terms of the variables studied before the start of the training. After the control period, there were no significant differences in dynamic and isometric strength, EMG parameters and hormonal concentrations in all the groups (Table 3). There were no significant differences in the body composition or body mass after training (Table 1). There were significant increases in the VO2peak in the EG (20.4±10.6%) and CG (22±10%) (p<0.05), and no modification was seen in the SG (5.7±7%) (Table 1). After the training period, a significant interaction was observed (time vs. group) on lower body 1 RM values (p=0.001). Post hoc tests demonstrated that all groups increased the lower-body dynamic strength (Fig. 1), but the increase in SG (67.6±17.1%) was significantly higher than CG (41.3±8.2%) and EG (24.7±8%) and CG significantly higher than EG (p<0.001). After training,

<table>
<thead>
<tr>
<th>Strength training</th>
<th>Endurance training</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week</strong></td>
<td><strong>Sessions</strong></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

RM: maximum repetitions; HRVT: heart rate at ventilatory threshold; min, minutes

Table 2 Complete strength and endurance training periodization.
The percent increase in maximal activation of vastus lateralis (1.1 ± 2% and 0.6 ± 1.2%) (p < 0.05). Regarding submaximal activation, significant decreases in the normalized electric activity of vastus lateralis were only seen in SG at 40, 60, and 80% of MVC (Fig. 5), rectus femoris at 60 and 80% of MVC (Fig. 6) and biceps femoris antagonist co-activation at 80% of MVC (Table 4).

The percent increase in maximal EMG activity of vastus lateralis (RMS values) before and after 12 weeks of training. CG, concurrent group; SG, strength group; EG, endurance group. (P < 0.01). *Significant difference from pre training values (P < 0.01). †Significant difference from EG.
In the present study, there were no significant interactions (time vs. group) for TT, FT, COR, TT/COR and FT/COR ratios (Table 5). There was a significant decrease in the FT concentrations after 12 weeks of training in EG (p<0.05), as well as a decreased FT approaching significance in CG (p=0.068), and decreased TT (p=0.06) and COR (p=0.052) approaching significance in EG. In addition, significant correlations between the increases in strength with the mean of TT (r=0.94; p<0.01) and the mean of TT:COR ratio along the study (r=0.93; p<0.01) were only seen in EG.

Discussion

The main finding of the present study was the presence of an interference effect on the gains in lower body-muscle strength observed in the concurrent group, which was not seen in the upper-body strength measurements in the same group. In addition, this interference effect occurred together with the different variations in EMG measurements obtained for the groups. Moreover, measurement of hormonal concentrations did not suggest any evidence of increased catabolic state. Furthermore, there was a significant relationship between strength improvements and basal testosterone and TT:COR ratio in the endurance group. Thus, the first hypothesis was rejected since our results showed an interference effect in the elderly subjects. Moreover, our second hypothesis was confirmed, since the maximal EMG adaptation was higher after training in SG, suggesting that the neural component may help to explain the interference phenomenon. Few studies have investigated concurrent training in elderly subjects, and, until now, no interference effect has been observed in this population [20, 23, 40]. In a study from Wood et al. [40], similar strength gains were observed in a comparison between strength and concurrent training after 12 weeks, 3 times a week, but with the concurrent group performing 50% less strength training volume (single set) compared to the strength group (2 sets). In another study, Izquierdo et al. [20] observed no interference effect when comparing 16 weeks of concurrent training with each modality performed once a week, and strength training twice a week (both 3–5 sets). Using the same volume in the concurrent and strength groups (3 sets), Karavirta et al. [23] showed that after 21 weeks of training, strength gains were the same in both groups. In the present study, although an interference effect was observed in the CG, this group had a similar or greater magnitude of strength gains after concurrent training (41%) in relation to the results of the above mentioned studies (21–44%), even though the number of training sessions were similar to those in the studies by Wood et al. [40], Izquierdo et al. [20] and Karavirta et al. [23] (36, 32 and 42 sessions respectively). These discrepancies could be explained by different initial levels of physical fitness among the subjects [1]. It is possible that by being less physically fit at the start of the training period, the sedentary elderly in the present study were able to obtain greater strength gains while, on the other hand, they were more susceptible to the interference effect when the endurance exercise was added.

Another possible cause of the interference effect observed in the present investigation may be the type of endurance exercise performed (cycle ergometer) [9, 28] and the timing of its execution, immediately before the strength exercises, since it has been demonstrated that a cycle ergometer exercise session can induce an acute decrease in lower-body force development due to the local fatigue [29, 36]. Possibly, there was concurrent recruitment of motor units used in both types of training, resulting in lower gains in dynamic strength in the CG. This would seem to be supported by the fact that the EG experienced a gain in dynamic strength (24.7%). Some authors have reported only a small improvement in lower-body strength in response to endurance exercise in the elderly.
training performed on a cycle ergometer when performed in combination with strength training [20,21], as well as when it is performed alone [39]. The same effects are not observed when aerobic training consists of running or jogging [25,32]. Thus, this would seem to suggest that the recruitment of high threshold motor units, responsible for higher force production, in the endurance training, especially at intensities near VT2, resulted in increased muscle strength in the EG, with local fatigue in these motor units, which led to decreased strength performance in the CG.

Although it has been suggested that the neural component of force production may be related to the occurrence of an interference effect [33], few studies have compared the adaptations of the EMG signal arising from strength and concurrent training [15,31] and no study has reported differences in the adaptations in maximal RMS values after training (strength vs. concurrent).

In the present study, significant increases in the amplitude of the EMG signal from the vastus lateralis and rectus femoris were observed in the SG after 12 weeks of training, while no modifications were observed in the CG and EG.

Our results are in accordance with the studies from Häkkinen et al. [13,14], which found a significant increase in the amplitude of the EMG signal after strength training, which suggests greater motor unit recruitment and a higher firing rate among the motor units. Furthermore, in the present study, the subjects in the SG experienced a significant decrease in muscle activation for the same absolute load (percent of MVC before training) on the vastus lateralis (40, 60 and 80%) and rectus femoris (60 and 80%), suggesting that for the same load, subjects needed fewer motor units after training [34], which is more economical at the neuromuscular level. The absence of similar adaptations in the CG suggests that the interference effect observed in the present study may be related to neural adaptations to strength training, with the endurance training session performed immediately before strength exercises negatively influencing such adaptations. In fact, a study by Lepers et al. [28], demonstrated that 30 min at 80% of maximal aerobic power resulted in decreases in isometric and concentric force, as well as decreases in the maximal EMG:M wave ratio, which suggests a central fatigue mechanism, indicating a reduction in the number of motor units recruited and/or lower firing rate during maximal effort. Thus, the fatigue imposed by endurance training may have prevented the neuromuscular system from developing the maximal capacity of voluntary recruitment of motor units and increases in the firing rate in the CG, resulting in lower strength development when compared to the SG. However, caution should be applied when dealing with the EMG data because it cannot detect activity at the level of single motor units and it underestimates the activation signal sent from the spinal cord to muscle [11]. Thus, it is possible that lower alterations to the maximal muscular activation in the CG might have occurred but remained undetected by the EMG, since it is unlikely that the strength improvements seen in the CG were exclusively the result of an increase in the muscle cross sectional area.

Besides mobilizing the energetic substrate after exercise, the endocrine system plays a regulatory role in muscular tissue repair and growth [26]. It has been suggested that modifications to the balance between anabolic (i.e., testosterone) and catabolic hormones (i.e., cortisol) may play a role in the interference effect seen in concurrent training, when such an effect is accompanied by increases in basal cortisol [4,5,25]. However, there were no significant modifications to basal hormones in the CG, indicating these subjects experienced no chronic catabolic state, and, consequently, the absence of any relationship between hormonal status and the strength gains found in the present study.
There was a significant reduction in FT, as well as a decreases in TT (3.7 ± 1.5 vs. 3.4 ± 1.6 ng/mL, p = 0.06) and cortisol (27.8 ± 3.0 vs. 24.5 ± 2.0 mg/dL, p = 0.052) approaching significance in the EG. These results, besides a reduction in FT approaching significance in the CG, suggest that the modifications observed might be related to endurance training, since no modification was observed in SG. Some studies have demonstrated that endurance athletes have a lower testosterone concentration than sedentary age-matched subjects [20, 30, 38]. Although there is no clear explanation for the reduction in the free testosterone observed in the EG in the present study, it is possible that the periodization performed, which included constant increases in volume and intensity, required more time for the endocrine system to adapt and reach an homeostatic state. In addition, modifications to resting testosterone levels may be transient and reflect the variation in volume and intensity of training and be explained by modifications to plasma volume [26, 30].

Several studies have demonstrated that testosterone levels and parameters related with this hormone (i.e., TT:COR ratio) are strongly related with muscle strength enhancement [1, 6, 12]. Surprisingly, in the present investigation, a significant correlation was found in the EG between the improvement in lower-body muscle strength and the TT concentration (r = 0.94) and TT: COR ratio values (r = 0.93). This may be explained by the fact that in the CG and SG the volume and intensity of the strength training was high, with 18 sets of knee extensions being performed until exhaustion (maximum repetitions) per week. Thus, the improvement in strength in the CG and SG is likely to be related with total load, as well the type of training performed (i.e., strength or concurrent) [7, 25].

Conclusions
To conclude, the differences in strength enhancement, resulting from strength and concurrent training suggests that endurance training performed before strength training can negatively interfere in the strength gains in elderly men, when the same muscle group is activated in both types of training. According to the mechanisms investigated, interference to neural adaptations seems to explain, at least in part, the interference effect seen in the concurrent strength and endurance training program. On other hand, the hormonal concentrations measured failed to suggest any increased catabolic state, reinforcing the hypothesis of a local neuromuscular and not a systemic interference effect, at least with the volume used in the present study. However, caution is necessary in the interpretation of the present results, since the small sample size and the absence of any morphological measure (i.e., magnetic resonance imaging) represent limitations. An important issue that must be highlighted is that, even though the increase in lower-body strength was at a lower magnitude than in the SG, the CG experienced a significant increase in this capacity (41%). From the perspective of promoting health, improvements in both strength and cardiorespiratory fitness are important and concurrent training seems to be the best strategy to enhance cardiorespiratory fitness, as widely shown in the literature, and achieve strength gains.

Acknowledgements

We thank specially to CAPES and CNPq government associations for their support to this project, to Mr. Guilherme Trindade, Mr. Steven Trangmar and Mr. Orlando Laitano for their help in the data collections and analysis. We also gratefully acknowledge to all the subjects who participated in this research and made this project possible.

References