Effect of adapted karate training on quality of life and body balance in 50-year-old men

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Background: Aging is associated with a decrease in physical skills, sometimes accompanied by a change in quality of life (QOL). Long-term martial arts practice has been proposed as an avenue to counter these deleterious effects. The general purpose of this pilot study was to identify the effects of an adapted karate training program on QOL, depression, and motor skills in 50-year-old men.

Methods and design: Fifteen 50-year-old men were enrolled in a one-year prospective experiment. Participants practiced adapted karate training for 90 minutes three times a week. Testing sessions, involving completion of the MOS 36-item Short Form Health Survey (SF36) and Beck Depression Inventory, as well as motor and effort evaluation, were done at baseline, and six and 12 months.

Results: Compared with baseline, participants had better Beck Depression Inventory scores after one year of karate training (P < 0.01) and better perception of their physical health (P < 0.01), but not on the mental dimension (P = 0.49). They also improved their reaction time scores for the nondominant hand and sway parameters in the eyes-closed position (P < 0.01).

Conclusion: Regular long-term karate practice had favorable effects on mood, perception of physical health confirmed by better postural control, and improved performance on objective physical testing. Adapted karate training would be an interesting option for maintaining physical activity in aging.

Keywords: karate, balance, training, sport, aging

Introduction

Within the context of aging and preserving quality of life (QOL), regular physical activity is indicated to maintain good health in the elderly population. Its beneficial effects are widely documented in the literature, and are widely recognized by older people. However, few studies in this domain have investigated the relationship between regular exercise, improvement of global physical state, and related QOL perception in healthy older adults.

Falls are a major threat in the elderly. Progressive decline in balance, flexibility, reaction time, and back strength have been associated with the risk of falls. Numerous studies have addressed the concern about falls and suggested that regular physical activity can improve physiologic parameters.

Martial arts, and tai chi in particular, have been proposed as a good way of maintaining the postural control essential for fall prevention. Tai chi is a traditional Chinese exercise with many benefits, including improved balance and postural stability, improved cardiorespiratory function, and is able to be undertaken by all age ranges.
groups. However, for better results, it has been suggested that this type of exercise be initiated in younger individuals (aged under 70 years) to promote regular, long-term practice which, in turn, may help to slow down the decrease in motor skills that leads to falls.12

Although a growing body of evidence has proposed tai chi as an ideal exercise for the elderly and people with cardiovascular risk factors, its effectiveness is still being debated. Furthermore, the slowness of this exercise has also been reported to be a reason for dropping out. It has also been shown that seniors could be interested in and improve their physical performance by practicing “hard” martial arts, such as tae kwon-do.19 This martial art, like karate, has all the advantages of tai chi and improves reaction time,20 a skill that decreases with age. Karate is a complete physical activity which makes the osteoarticular and muscular systems work as a whole. Additionally, other martial arts might be seen as fall prevention tools by the learning of safe fall techniques.21 In light of these issues, it has been hypothesized that karate might be a suitable physical activity for middle-aged people in the long-term prevention of falls.

As far as we know, no prospective study has evaluated the effectiveness of martial arts for a long period (more than six months), although beneficial effects of long-term practice of martial arts have been reported. The present pilot study aimed to document the long-term effects of one year of regular practice of adapted karate training in a group of healthy men in their sixth decade of life.

Methods and study subject

Subjects and study design

In this one-year prospective study, participants attended adapted karate training for at least three weekly sessions of 90 minutes each for 12 months. Physical, psychologic, and QOL outcomes were assessed at baseline, and after six and 12 months. The first session (baseline) took place before the adapted karate training began. The second and third sessions were at six and 12 months, respectively, after the first training session. The study was approved by the Ethics Committee of the Centre Hospitalier Territorial du Taone, French Polynesia and the Ethics Committee of French Polynesia. All study subjects provided written consent.

Twenty-two healthy 50-year-old male volunteers enrolled in the study. Of these, 16 participated in a second testing period, and 15 in a third testing period. The volunteers were required to participate in training three times a week. Inclusion criteria were age at least 50 years, working, and not engaged in regular physical training. Regular physical activity was defined as exercise performed at least twice a week for more than 30 minutes during the past year, according to which one volunteer was excluded at the baseline interview. Exclusion criteria were any existing cardiac, respiratory, rheumatologic, neurologic, or metabolic disease, which were screened for at the baseline evaluation by blood investigations and clinical examination.

The study was advertised by an information sheet placed in various locations, ie, hospitals, schools, and government work places. At the end of the recruitment period, no women had expressed a wish to participate, and due to the pilot nature of the study, we decided to limit the sample to 20 participants.

Adapted training

To ensure “adapted” training, we removed all causes of shock (eg, avoiding antiphysiologic postures and techniques), adapted progression (eg, training at a slow cadence, a push-attack approach), and optimized coaching according to the ability of each trainee. An adapted karate training session involved 15 minutes of warmup exercise, one hour of training, revision of past acquired skills, discovery, learning, and integration of new elements, with 15 minutes of stretching and cooling down. Training content, developed for the elderly by an expert teacher (third Dan), was provided by four different teachers, according to a standardized protocol. All of the teachers were trained together to teach this adapted form of karate during training sessions held four times a week.

Evaluation

All participants completed baseline measurements at least one week before training began. In addition to evaluation of physical, psychologic, and QOL parameters, participants underwent blood analyses and clinical examination at baseline. Blood analyses allowed us to measure fasting blood glucose, lipid profile, and uric acid concentrations, and confirm eligibility. All clinical measurements were undertaken at the Centre Hospitalier Territorial du Taone in French Polynesia. Two other evaluations (at six and 12 months) included all tests performed at the baseline, except for the blood investigations.

Outcome measures

QOL, the primary outcome of the study, was assessed with a validated French version of the MOS 36-item Short Form Health Survey (SF36). This tool comprised 36 items representing eight dimensions of health-related QOL, physical activity, functional status, pain, general health, vitality, social functioning, emotional status (REt), mental health, and health transition. We also calculated mental
composite score and physical composite score, two scores developed by principal component analysis.23 This self-administered questionnaire provided scores for the overall health of participants.

We also used the Beck Depression Inventory (BDI) to evaluate mood status in our subjects. This self-administered 21-item questionnaire has been tested for reliability, specificity, validity, and sensitivity, and has been used in patients as well as healthy populations.24 Scores of 10–18 indicated mild to moderate depression, and scores ≤9 indicated normal mood range.

Specific motor functions were quantified by computerized testing. Reliability, validity, specificity, and sensitivity of this testing procedure have been verified in several populations.25–27 Hand coordination and postural stability were evaluated with the CATSYS system (Danish Product Development Company, Snekkersten), with coordination testing performed using the right and left hands. Reaction time measurement is a test whereby the evaluator asks the subject to press a button following a sound stimulus from which mean reaction time is obtained, with larger values indicating poorer performance. Finger tapping measures maximal velocity of rapid forefinger movement. Subject performances were examined at a constant slow (1 Hz) and rapid (2.5 Hz) beat. Subjects did the same test at accelerating rhythms from 1.6 Hz to 8 Hz. The pronation/supination test was the last evaluation of hand coordination to be conducted. This experiment was conducted at a constant slow (1 Hz), rapid (2.5 Hz), and accelerated beat (6–7.5 Hz). The parameters calculated for reaction time, finger tapping, and pronation/supination tests gave an estimated value of overall hand coordination. In the second and third of these three tests, information is collected for rhythmic regularity, precision, and maximal frequency. The first test evaluates the rhythmic regulation to keep up precision. Values are always positive, with the smallest values indicating better regularity. The second measure is the mean of accuracy of contact in relation to a metronome beat. The value nearest zero indicates best precision. The last parameter recorded is the maximum frequency obtained, and larger values indicate better performance.

Postural sway was evaluated by a platform containing three orthogonal strain-gauge devices that measure involuntary postural oscillations according to a Cartesian axis. For each subject, postural sway was evaluated for 75 seconds under four different conditions, ie, eyes opened, eyes closed, and with and without a soft foam pad under the feet.28 Motor skills were assessed according to the same specific sequence, ie, reaction time (right and left hands), sway measurements (eyes open and closed), tremor evaluation (right and left hands), pronation/supination (right and left hands) and, finally, finger tapping (right and left hands). This sequence was developed to minimize the influence of stress. The duration of each test and metronome beat was the same as that used previously by Després et al.28 We statistically analyzed parameters estimated by CATSYS, except for harmonic and tremor indices for which we applied the modification proposed by Edwards and Beuter.29 For each test, records were obtained after the training period in order to ensure that performance was not affected by comprehension of the task.

Effort was tested on an ergometer bicycle (Schiller ergometer CE 0.124, Baar) according to a triangular protocol with a prior five-minute warmup period. This protocol implies an increment of developed power of 25 W at 1.5-minute intervals, until the theoretical maximal frequency (220) is reached or stoppage criteria appeared. The test provides information on a range of parameters, including duration of effort, maximal power developed, maximal heart rate, and individual effort profile.

Statistical analysis
All continuous variables of the subsample for which clinical data were available are presented as arithmetic means accompanied by their standard deviations (mean ± SD). The means for continuous variables were compared by conventional t-test, with Fisher’s Exact tests comparing proportions. When the distribution of variables was not normal, the parameters of dispersion were presented as median and interquartile ranges, with comparisons by the Mann–Whitney U-test.

We analyzed the data as repeated-measures outcomes according to the mixed model approach.30 The covariance structures applied in our models were of the first-order autoregressive form, and convergence was achieved for all models. In this analysis, we compared changes in continuous variables (mean function scores) from baseline to six and 12 months. Multivariate analysis was performed, with potential confounders retained in univariate analyses. The preselection criteria of potential confounders were an association with the dependent variable having a $P$ value < 0.20. We considered a confounding effect if the $\beta$-coefficient in the model showed a change of 10% or more after adjustments for a potential confounder.

Post hoc power sample size calculation, using the G power, was also calculated at the time of the analytic phase. For an effect size calculated31 ($F^2 = 0.37$) based on one of our main outcomes, BDI, a sample size of 20, and $\alpha = 0.05$.
(two-tailed). The power was 86.1%, which was considered to be adequate statistical power. All statistical analyses were performed using SAS software, (version 9.1; SAS Institute Inc., Cary, NC) and the level of statistical significance was set at $\alpha = 5\%$.

**Results**

Of the 21 men tested at baseline, 15 completed the study. The total attrition rate was 28.5%. Three men dropped out before the end of the first month of the study. Three other participants left the karate group before the second testing period. The main reasons for attrition were poor motivation and a busy schedule. The demographic characteristics of the six men who withdrew did not differ significantly from those who completed the study.

Table 1 shows the characteristics of the participants at baseline. They were comparable for all characteristics except for educational level (Table 1). In terms of marital status, 91.8% were married, and 8.89% were living in common law marriages.

For depression, we observed a significant halving of BDI score at the second ($4.9 \pm 1.0, P < 0.01$) and third ($4.9 \pm 1.1, P < 0.01$) evaluations compared with baseline ($9.8 \pm 1.1$). No differences between the second and third evaluations were detected, even after adjustment for education and age.

For health dimensions measured by the SF36, only scores related to physical health perception changed significantly during the study (see Figure 1). Indeed, PCS increased significantly from baseline to the third evaluation ($29.8 \pm 1.1$ versus $34.7 \pm 1.1, P = 0.01$) as did physical functioning ($81.85 \pm 1.9$ versus $87.99 \pm 1.9, P = 0.02$), body pain ($69.85 \pm 2.2$ versus $84.52 \pm 2.4, P = 0.04$), general health perception ($13.92 \pm 0.7$ versus $16.40 \pm 0.7, P = 0.01$), and vitality ($61.04 \pm 1.6$ versus $69.12 \pm 1.6, P < 0.01$). However, we did not see any changes in mental health status dimension evaluated by the SF36, such as the mental composite score ($50.85 \pm 1.3$ at baseline versus $50.16 \pm 1.3$ at 12 months, $P = 0.67$) and other health status measures, such as social functioning (baseline versus third evaluation at 12 months, $P = 0.77$), REt (baseline versus third evaluation, $P = 0.50$) and mental health (baseline versus third evaluation, $P = 0.95$). All results include adjustment for age.

During the study period we observed some differences in manual skills measured by reaction time, pronation, supination, and finger tapping tasks, as reported in Table 2. Indeed, we noted significant improvement in left hand reaction time for the participants, all of whom were right-hand dominant. Moreover, maximum frequency in pronation and supination was increased in both hands. Similar results were obtained with left hand finger tapping.

Sway performance was evaluated in all participants. As seen in Table 3, significant improvements were only evident in the eyes-closed condition. Improvements in velocity and stability were seen.

For effort testing, we observed significant improvement in duration of effort from baseline to the third evaluation at 12 months ($10.86 \pm 0.5$ versus $12.23 \pm 0.6$, respectively, $P = 0.04$) and for MPD from baseline to the second evaluation at six months ($198.01 \pm 10.01$ versus $214.80 \pm 10.24$, respectively, $P = 0.01$). However, we did not detect any changes in MPD, MCF, and individual effort profile. All results for effort testing were adjusted for body mass index.

**Discussion**

This pilot study of 15 men in their sixth decade of life suggests that regular, adapted karate training over a one-year period might have a positive influence on several QOL parameters and motor skills which decline with increasing age. In these men, the beneficial effects of regular karate practice were apparent within six months of starting training and maintained at one year.

Each physiologic parameter measured in this study was improved in our participants compared with baseline, suggesting a positive effect of training. In particular, we observed improvement in all parameters that are important in fall reduction, notably postural sway and reaction time. The latter seems to be inherent in karate practice, because similar results were obtained among young karate athletes who demonstrated better scores on reaction time tasks and anticipatory skills than novice individuals. For postural sway, after a one-year training period and compared with baseline, the participants showed improvement of sway velocity and sway intensity, both of which are important in postural control and decline with increasing age. In other words, among adults with a low risk of falling, this intervention improved sway movements associated with risk.

| Table 1 Demographic and social characteristics of participants |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (years)* | N | Median | IQR | Minimum | Maximum |
| Education (years)† | 15 | 03.00 | 05.00 | 03.00 | 14 |
| Weight (kg) | 15 | 85.70 | 24.30 | 57.70 | 96.50 |
| Height (cm) * | 15 | 161.34 | 0.07 | 01.65 | 01.90 |
| Body mass index (kg/cm²) | 15 | 26.73 | 05.11 | 21.19 | 32.09 |

Notes: *Mean ± standard deviation; †0 = high school, <0 = undergraduate, >0 = university.
Abbreviation: IQR, interquartile range.
of falling. These results warrant further investigation among older adults to measure more precisely the effectiveness of this intervention. The necessity of preventing falls and their sequelae among the elderly is becoming increasingly important. Accordingly, adapted karate training appears to be a promising exercise that is as effective as other martial arts currently recommended for seniors.

Concomitant with the improvement in physical parameters, the results of this study indicated a clear improvement in QOL and mental health status. Similarly

Table 2 Changes in manual skills between baseline and two subsequent time points

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Baseline Mean ± SD</th>
<th>Six months Mean ± SD</th>
<th>12 months Mean ± SD</th>
<th>P value (B versus 6 months)</th>
<th>P value (B versus 12 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH (sec)</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.71</td>
<td>0.86</td>
</tr>
<tr>
<td>LH (sec)</td>
<td>0.20 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.01*</td>
<td>0.01*</td>
</tr>
<tr>
<td>Pronation and supination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH precision (sec)</td>
<td>0.01 ± 0.02</td>
<td>-0.02 ± 0.02</td>
<td>-0.26 ± 0.02</td>
<td>0.04*</td>
<td>0.11</td>
</tr>
<tr>
<td>RH regularity*</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.37</td>
<td>0.96</td>
</tr>
<tr>
<td>RH max (Hz)</td>
<td>5.43 ± 0.32</td>
<td>6.07 ± 0.30</td>
<td>6.10 ± 0.32</td>
<td>&lt;0.01*</td>
<td>0.04*</td>
</tr>
<tr>
<td>LH precision (sec)</td>
<td>-0.03 ± 0.02</td>
<td>-0.04 ± 0.02</td>
<td>-0.36 ± 0.02</td>
<td>0.41</td>
<td>0.66</td>
</tr>
<tr>
<td>LH regularity*</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.15</td>
<td>0.82</td>
</tr>
<tr>
<td>LH max (Hz)</td>
<td>5.07 ± 0.24</td>
<td>5.85 ± 0.23</td>
<td>5.88 ± 0.26</td>
<td>&lt;0.01*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Finger tapping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH precision (sec)</td>
<td>-0.09 ± 0.01</td>
<td>-0.06 ± 0.01</td>
<td>-0.06 ± 0.01</td>
<td>0.01*</td>
<td>0.10</td>
</tr>
<tr>
<td>RH regularity</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.03*</td>
<td>0.08</td>
</tr>
<tr>
<td>RH max (Hz)</td>
<td>6.72 ± 0.27</td>
<td>6.35 ± 0.27</td>
<td>6.23 ± 0.30</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>LH precision (sec)</td>
<td>-0.09 ± 0.01</td>
<td>-0.06 ± 0.01</td>
<td>-0.06 ± 0.02</td>
<td>0.09</td>
<td>0.28</td>
</tr>
<tr>
<td>LH regularity*</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.49</td>
<td>0.42</td>
</tr>
<tr>
<td>LH max (Hz)</td>
<td>6.09 ± 0.39</td>
<td>6.98 ± 0.39</td>
<td>6.85 ± 0.43</td>
<td>0.10</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Notes: All results were adjusted for the dominant hand and age. *P values which reached statistical significance (P < 0.05).

Abbreviations: B, baseline; LH, left hand; RH, right hand; SD, standard deviation.
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Table 3 Change in sway skills between baseline and two subsequent time points

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Baseline Mean ± SD</th>
<th>Six months Mean ± SD</th>
<th>12 months Mean ± SD</th>
<th>P value (B versus 6 months)</th>
<th>P value (B versus 12 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eyes open</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm)</td>
<td>5.6 ± 0.5</td>
<td>6.0 ± 0.5</td>
<td>6.1 ± 0.5</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Transversal (mm)</td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>0.46</td>
<td>0.57</td>
</tr>
<tr>
<td>Sagittal (mm)</td>
<td>4.1 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.6 ± 0.5</td>
<td>0.43</td>
<td>0.40</td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>316.6 ± 40.0</td>
<td>315.2 ± 38.6</td>
<td>321.3 ± 8.0</td>
<td>0.96</td>
<td>0.80</td>
</tr>
<tr>
<td>Velocity (mm/sec)</td>
<td>11.4 ± 0.9</td>
<td>10.6 ± 0.9</td>
<td>10.5 ± 0.9</td>
<td>0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>Intensity (mm)</td>
<td>4.4 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>0.97</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Eyes closed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm)</td>
<td>6.71 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>0.05*</td>
<td>0.19</td>
</tr>
<tr>
<td>Transversal (mm)</td>
<td>3.9 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>3.3 ± 0.4</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>Sagittal (mm)</td>
<td>4.6 ± 0.5</td>
<td>4.1 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>0.12</td>
<td>0.40</td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>507.9 ± 87.5</td>
<td>404.9 ± 84.1</td>
<td>399.2 ± 89.3</td>
<td>0.09</td>
<td>0.22</td>
</tr>
<tr>
<td>Velocity (mm/sec)</td>
<td>17.5 ± 1.9</td>
<td>14.4 ± 1.8</td>
<td>14.4 ± 1.9</td>
<td>0.02*</td>
<td>0.01*</td>
</tr>
<tr>
<td>Intensity (mm)</td>
<td>6.2 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>0.01*</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

Note: *Statistically significant at P < 0.05.

Abbreviations: B, baseline; SD, standard deviation.

convincing results have been obtained in epidemiologic investigations of sustained practice of other martial arts. Tai chi was associated with enhanced well-being and perceived health by traditional Chinese practitioners.6,13 However, we did not notice any significant changes in mental health dimensions evaluated by the SF36 or other parameters, such as social functioning and REt. Social functioning measures social limitations related to physical and psychologic problems, while REt assesses difficulties in daily activities stemming from emotional problems.23 However, the SF36 was recently found to be influenced by sociodemographic status in European patients.35 Consequently, our results are probably related to the characteristics of the study participants, who were all employed and did not report major changes in their lives during the study year. Moreover, our data in this respect are very similar to those recorded in the general population for the same age group (data not included).23 Nevertheless, at one-year follow-up, we noted significant improvement in mood for our participants.

These results echo those obtained on BDI testing for which participants showed an improved score at six months which was maintained after one year. Similar positive effects have been observed among adults practicing other martial arts.36 This innovative pilot study corroborates the results obtained in other populations practicing tai chi. Our findings also suggest that adapted karate training may have similar beneficial effects as tai chi, which is already very popular among the elderly worldwide.

As reported recently in a randomized study of regular tai chi in diabetics, many aspects measured by the SF-36, such as physical functioning, were improved.38 However, it has been suggested that tai chi may not be intensive enough to produce metabolic changes. Karate, as a “hard” martial art, might be a better alternative in this setting.

However, our study suffers from some limitations inherent in the study design. The size and nature of the sample (ie, males only) decrease the external validity of the study and prevent any generalizations being made from our findings. More importantly, the lack of a comparison group (without intervention) suggests that our results might be a natural aging effect and could explain the plateau observed between the second and third evaluation. Consequently, these results should be considered as encouraging preliminary data, and the starting point of a new 18-month intervention study in a larger sample of population.

In conclusion, the results of this pilot study indicate that adapted karate training may contribute to slowing the inexorable process of aging. Karate training seems to enhance psychologic and social dimensions as well as physiologic performance. In terms of public health promotion, adapted karate training would be an interesting option to maintain physical activity during the aging process.
Acknowledgments

This study was funded by the French Polynesian Government contract EPAP. The authors thank the nurses of the Cardiology Service of Centre Hospitalier Territorial du Taone for their cooperation, as well as the two karate professors for their active participation in adapted karate training. The authors also thank the CHUQ Research Center for loaning the CATSYS System, and are also grateful for the financial support provided by the Canadian Institutes for Health Research Institute for Aboriginal People’s Health fellowship program. This work was presented in part as a poster at the Second Rencontres de l’Association Francophone pour la Prévention-Réadaptation Cardiovasculaire held in Paris, France, in October 2006.

Disclosure

The authors report no conflict of interest in this research.

References


