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Taekwondo training reduces blood catecholamine levels and arterial stiffness in postmenopausal women with stage-2 hypertension: full study protocol and statistical analysis plan

NCT#03544307

Full study protocol:

Subject Characteristics

All procedures employed in this study were conducted and performed according to the protocols approved by Kosin University's Institutional Research Board (KU IRB 2015-0019) and carried out in accordance with the Declaration of Helsinki. This study was also registered with clinicaltrials.gov (NTC03544307). Twenty postmenopausal, stage-2 hypertensive women (70±4 years old**)** were recruited for this study and informed consent was obtained. Participants were screened by physicians of Kosin University and excluded if participants were obese (body mass index >30 kg/m2), smokers, had heart disease, renal disease, psychiatric conditions as assessed by medical history, and/or taking medications or hormone therapy in the year before the study. In addition, all participants were sedentary (<1 h of regular exercise per week in the previous year). Allocation was stratified for systolic BP (<140 or ≥140 mmHg), and sequence was generated by a computer-based number (figure 1). Subjects were randomly divided into two groups: 1) Taekwondo training (TT, n=10) and 2) Control (CON, n=10) (Figure 1). Subject characteristics can be seen in Table 1. Measurements were obtained at baseline and at 12 weeks during the same time of the day (±1 hour) in the morning and after an overnight fast. Subjects abstained from caffeinated drinks and alcohol between 48 and 72 hours before baseline testing and after the last exercise session. Cardiovascular measurements were collected in a quiet temperature-controlled room (22-24 ^{0}C) after at least 10 minutes of rest in the supine position. Participants were instructed not to alter their regular lifestyle habits during the study period (verified through food/physical activity logs).

Taekwondo Training Program

According to the ACSM guidelines for exercise testing and prescription[27], the current investigation applied a 12-week Taekwondo training protocol of 3 times/week, 60 min/per session for the TT group. Exercise intensity was set at 30-40% heart rate reserve (HRR: maximum heart rate – resting heart rate) for the first four weeks and gradually increased up to 50-60% HRR during the last four weeks of training

(Table 2). Heart rate was measured by Polar RS400 heart rate monitor (Polar Electro, Kempele, Finland) in order to monitor the exercise intensity. The participants in the CON group maintained their normal life without any manipulation but were present in the laboratory at the same frequency and duration of the TT group throughout the whole study.

Blood Sampling

The subjects were asked to report to the laboratory fully rested and fasted from 8 pm the day before the blood sampling. 10 ml of blood were collected from the brachial vein using vacutainer and needle (Bom Medrea Co, LTD, Phnom Penh, Cambodia) by a trained phlebotomist before and after Taekwondo training. Blood serum was separated by centrifuge at 3,000 rpm for 10 minutes. Blood catecholamine levels of epinephrine and norepinephrine were analyzed by using commercially available ELISA kit (Abnova, Taipei City, Taiwan).

Arterial Stiffness

Brachial-ankle pulse wave velocity (baPWV) was measured using non-invasive arterial tonometry with SphygmoCor (AtCor Medical, New South Wales, Australia) and analysis software (version 8.0, SphygmoCor Cariovascular Management Suite) for measurement of baPWV(m/s), an indicator of arterial stiffness. Two measurements were collected at each time point and averaged as previously described[23, 24].

Muscle Strength

Leg muscle strength was assessed by the one repetition maximum (1RM) test using a leg extension machine (Cybex 6000; Lumex, Albertson, NY). Subjects were familiarized with the exercise and lifting technique before 1RM measurement, which was achieved within five attempts. The 1RM was considered the highest weight lifted using the proper form. Handgrip muscle strength was measured as the highest of three maximal voluntary contractions using a handgrip dynamometer (Jamar, Bolingbrook, IL).

Statistical analysis plan:

All parameters were normally distributed as shown by the Shapiro-Wilk test. Differences at baseline between groups were evaluated using unpaired t tests. Data were analyzed by SPSS statistical software (BM SPSS Analytics version 23.0; IBM, Armonk, NY). A two-way ANOVA with repeated measures [groups TT and CON] x time [before and after 12 weeks]) was used to determine the statistical significance of differences between pre-training and post-training. If significance was found, paired t-tests were used for *post hoc* comparisons. Independent samples t-test were also used to examine the baseline differences between the TT and CON groups. The significance was defined *a priori* as $P<0.05$. Data were expressed as a mean ± standard error. G*Power 3.1 power analysis software (Universität Kiel, Germany) was used to determine the appropriate number of subjects. A power analysis calculation determined a minimum sample size of 20 (10 each group) would allow the observation of a difference of 3% to 5% between the group (TT vs CON) on the primary study outcome variable of baPWV with a power of 80%.