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**Title:** Are 30 minutes of rest between two incremental shuttle walking tests enough for cardiovascular variables and symptoms to return to baseline values?

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**Body:** Background: The Incremental Shuttle Walking Test (ISWT) is commonly performed twice for purposes of reproducibility, with in general, 30 minutes of rest between the tests. However, it is unknown if the 30 minutes' rest is sufficient for stabilization of cardiovascular and symptomatological variables. Aim: To investigate if 30 minutes of rest between two ISWTs are enough for cardiovascular and symptomatological variables to return to baseline values in healthy subjects of different ages. Methods: 457 healthy subjects (154 men, 63[45-70] years, 27[24-30] kg/m<sup>2</sup>) were assessed and separated into quartiles according to their age: Q1 (18 to 45 years, n=117), Q2 (46 to 63 years, n=116), Q3 (64 to 70 years, n=121) and Q4 (71 to 83 years, n=103). Two ISWTs were performed with at least 30 minutes of rest in between, and heart rate (HR), blood pressure (BP) and symptoms of dyspnea and fatigue were assessed before and after the tests. Results: The HR before the ISWT was higher in the second test compared to the first one in the whole group and in all subgroups (p<0.0001 for all). The systolic BP was higher before the second test only in the whole group (p=0.04). Regarding symptoms, fatigue showed statistical significance only in Q1 (p=0.02), being higher before the second test compared to the first. Diastolic BP and symptoms of dyspnea were similar before the two ISWTs in the whole group and in all subgroups. Conclusions: 30 minutes of rest between two ISWTs are not enough for the cardiovascular system to return to baseline values in healthy subjects, regardless of age. For perceived symptoms of dyspnea, this amount of rest seems to be enough.