Purpose: (1) To determine the reliability and cardiopulmonary effects of the Two-Minute Walk Test (2MWT) among older adults using the Alter-G anti-gravity treadmill; (2) to determine the effects on functional balance after the use of the Alter-G. Number of Subjects: Eleven (M=5, F=6) elderly residents (mean age=84.7 ± 4.32 yrs) from an assisted living facility. Materials/Methods: Subjects provided informed consent, completed a cognitive exam, medical history, Par-Q risk, and physician approval. Baseline over-ground 2MWT, Four Square Step Test (FSST), and Frailty and Injuries: Cooperative Studies of Intervention Techniques (FICSIT) were administered on each subject with systolic blood pressure (SBP), heart rate (HR), and oxygen saturation (SaO2) levels obtained prior to, during, and after each 2MWT. Subjects were assisted into the Alter-G for a familiarization session of treadmill ambulation with progressively increased body weight support to 60%. Participants then completed randomized Trials 1 and 2 on two separate days. Trial 1 consisted of completion of two Alter-G 2MWTs, FSST, and the FICSIT. Trial 2 consisted of two over-ground 2MWTs, FSST, and the FICSIT. An Intraclass Correlation Coefficient (ICC) was computed to determine the reliability of the 2MWT on the Alter-G and over-ground. Repeated measures ANOVA analyze differences in distance walked, HR, SBP, and SaO2 on the Alter-G and over-ground 2MWTs. Results: Two-minute walk over-ground distance averages were 266.8’ vs. 216.5’ on the Alter-G treadmill. ICCs indicated a higher test-retest reliability for the 2MWT when performed over-ground (0.971, p<0.001) versus on the Alter-G treadmill (0.686, p<0.050). No significant changes in either the FSST or FICSIT were found following either the over-ground or the Alter-G 2MWTs. Results of the repeated measures ANOVA revealed a significant increase in HR within trials for the 2MWT performed over-ground on day one (p<0.001) and day two (p<0.048). Significant differences in SaO2 were also found on day one (p<0.005) and day two (p<0.012), but no changes in SBP were noted. No significant changes in HR, SBP, or oxygen saturation were found between trials for the 2MWT when performed on the Alter-G. Conclusions: Physiological and distance walked results of the 2MWT performed over-ground versus the Alter-G are not comparable. Greater distances were recorded more consistently on the over-ground test and produced more physiological changes in subjects’ HR and SaO2 levels. Neither test significantly affected static or dynamic balance outcomes. Clinical Relevance: The over-ground 2MWT may be easier to administer in the clinic because it requires less time, less equipment, and results in better reliability than the same test performed on the Alter-G. However, this data also shows that there was no significant change in balance post Alter-G 2MWT among those in assisted living facilities. From this, we infer that in the elderly, fall risk neither increases or decreases after a 2-minute Alter-G locomotion session and appears safe regarding the subject’s measured balance.