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Oxygen uptake kinetics differentiates two from six minute walk tests in lower extremity prosthesis users

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The purpose of this study was to evaluate oxygen uptake kinetics and biomechanics during two and six minute walk tests in individuals with lower limb prosthesis (iLLA) and unimpaired persons. Participants performed both 2MWT and 6MWT, oxygen uptake (VO_2), heart rate (HR) and temporal-spatial parameters were recorded. Repeated measures factorial ANOVAs analyzed differences between tests and groups, Alpha was set at 0.05. There were no significant differences in VO_2 or HR between iLLA and unimpaired groups at any time point during either walk ($p > 0.05$), and neither group achieved steady state during the 2MWT, whereas steady-state HR appeared after minute 4 of the 6MWT in both groups. iLLA walked significantly less distance ($p < 0.05$) but had similar cadence and total steps during each test compared with unimpaired ($p > 0.05$). iLLA also had significantly greater stance ratios and shorter stride length compared with the unimpaired group ($p < 0.05$) and stride length was also shorter in iLLA during the 6MWT compared to the 2MWT ($p < 0.05$). The 2MWT is a strong predictor of 6MWT in the unimpaired group ($r = 0.76$, $p = 0.001$) and stronger in those using prostheses ($r = 0.94$, $p = 0.001$). Although 2MWT was a strong predictor of 6MWT performance in both groups, marked physiological and biomechanical differences were observed. Our findings do not support use of 2MWT as a proxy for 6MWT in lower limb prosthesis users.

Timed walking tests evaluate mobility of individuals with lower limb amputation (iLLAs) quite well. The two-minute walk test (2MWT) and six-minute walk test (6MWT) evaluate maximum distance a person with lower limb prosthesis walks over two or six minutes respectively[1–3]. Although these tests may not be sensitive enough for assessing effect of prosthetic changes[4,5], they are useful in determining mobility and perhaps functional capacity. Duration appears to be the distinguishing factor between the two tests, however, the increased duration of the 6MWT places additional physiological strain on the individual beyond that required for the 2MWT[6]. Still, some scholars have suggested using the 2MWT as a direct replacement of the 6MWT[7]. Although the 2MWT distance was predictive of the 6MWT distance ($R^2 = 0.91$)[7], the claim that the 2MWT can be used to “gain the same knowledge” as that of the 6MWT may be debatable[7].

The 6MWT was originally developed as an alternative to the longer 12-min walk test which was an alternative to the Cooper 12-min run test[8]. The Cooper test was strongly correlated with maximal volume of oxygen uptake (VO_2) max (0.89) and was recommended as a suitable alternative for measuring aerobic capacity. Thus, the American Thoracic Society guidelines for assessing cardiorespiratory endurance and aerobic capacity have recommended the 6MWT as a suitable noninvasive alternative of the aforementioned tests[6,9]. Hence, although the 2MWT is wonderful at testing walking function (mobility), it may not be long enough to determine functional capacity in iLLAs.

The functional physical capacity of prosthesis users is a reflection of their aerobic capacity to perform activities of daily living[10]. The simple act of walking is effective at eliciting an increase in the cardiovascular and skeletal muscle systems. Volume of oxygen uptake (VO_2) measurement provides an index of a person’s ability to transport oxygen to the working muscle. Measurement of VO_2 has helped to better understand human locomotion and gait pathology[11–16], and there is a linear relationship between work rate and oxygen uptake[17–19]. However, similar to resting oxygen uptake[20], people do not immediately achieve “steady-state” oxygen uptake when beginning to walk. During continuous exercise, “steady-state” occurs when the body’s demand for oxygen is met[21]. Steady-state assumes invariant VO_2, carbon dioxide (CO_2) and heart rate (bpm), and typically occurs

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within 3 min at a constant moderate work load[22,23]. Various factors, including delays in metabolism, age, fitness, disease, and familiarity with testing may prolong attainment of steady-state oxygen uptake[24]. Hence, temporal dynamics of VO_2 during walking tests may offer an index of a person's fitness level.

Functional (aerobic) capacity is typically measured through a staged VO_2 max test[19]. However, the test is physiologically demanding and necessitates costly equipment. Of practical timed walking tests, the 6MWT is a validated alternative assessment of functional capacity. The test is correlated with VO_2 max in a variety of populations[25–27], and has been used to determine functional capacity of iLLAs[28,29]. To our knowledge, comparison of VO_2 kinetics of prosthesis users during the 2MWT and 6MWT has yet to be explored. A better understanding of oxygen uptake dynamics may better distinguish the two tests from one another, and elucidate steady-state VO_2 dynamics in these individuals. Therefore, this study was undertaken to evaluate the oxygen uptake kinetics during the two and six minute walk tests in iLLAs. In addition, we evaluated oxygen uptake kinetics during tests between amputee and unimpaired persons. This was done to examine if prosthesis use would increase time to steady state.

Methods

Participants

The study was approved by the Texas A&M University San Antonio Institutional Review Board (Log # 2021-38) with participants signing an informed consent form prior to study commencement. All procedures were performed in accordance with university guidelines, regulations and the Declaration of Helsinki. Twenty-two lower limb prosthesis users (iLLA) and 17 unimpaired persons participated in this study (Table 1). The selection criteria included people with a lower limb amputation who could ambulate with their prosthesis without an assistive device, and excluded those who might be pregnant. All participants were asked to not eat a heavy meal four hours prior to testing, not to exercise, and maintain hydration prior to data collection. Height was measured wearing prostheses without shoes to the nearest 0.1 cm using a stadiometer (Seca® 213, Hamburg, Germany). Body mass was then measured to the nearest 0.1 kg using a digital scale (BOD POD®, COSMED, USA Inc., Concord, CA, USA). Body mass index was determined by mass divided by stature squared (kg/m^2). All measurements were carried out according to manufacturer instructions.

Testing protocol

Each participant reported to the lab and were counter-balanced to first perform either a 2MWT or 6MWT, and within one week return to the lab to perform the other. Participants were instructed on the procedures for the walk test on each the testing day. They were told to walk as fast as possible in a safe manner while covering as much ground as possible. Participants were told they were permitted to stop but that time would continue and that they were to resume when able to do so. At the end of the walk, they were instructed to stop as quickly as possible. Participants were fitted with a Polar heart rate monitor chest strap (Polar, Kempele, Finland) to measure heart rate and a Cosmed K5 portable metabolic analyzer (COSMED, Rome, Italy), which was calibrated following manufacturer's guidelines.

Participants were also fitted with Opal wearable triaxial Inertial Measurement Units (IMU) (Clario APDM, Philadelphia, PA) on each foot and ankle, as well as the lumbar and chest to collect cadence, gait speed, stance ratio, and stride length[30–33]. These data were collected at 128 Hz and calibration was performed for each participant prior to data collection.

Participants sat for three minutes while resting heart rate (HR) and oxygen consumption (VO_2) was collected. After the three minutes, participants stood behind a start line, with the metabolic analyzer marked to indicate the start of the walk. Two investigators followed the participant to avoid pacing, one keeping time and filming (to later manually count steps with a hand tally counter) and the other with a measuring wheel to mark distance at 1 and 2 min of the 2 min walk, and 2 and 6 min of the 6 min walk. Participants walked along a flat, indoor level floor with a right turn approximately every 50 m for either two or six minutes depending on the test. The metabolic analyzer was marked to indicate the end of the walk, and a rating of perceived exertion (RPE) (Borg's 6–20 scale) was recorded[34]. Distance walked was recorded and metabolic data was analyzed OMNIA software (COSMED, Rome, Italy). Gait data was analyzed using Mobility Lab software (Clario APDM, Philadelphia, PA) interfaced with the Opal sensors.

Characteristics	Total (N = 39)	Unimpaired (n = 17)	Prosthesis (n = 17)
Age (y)	55.0 ± 14.8	58.8 ± 14.3	51.1 ± 14.5
Height (cm)	169.6 ± 9.6	168.2 ± 10.4	170.9 ± 7.8
Mass (kg)	89.0 ± 19.7	84.5 ± 19.5	93.4 ± 17.7
BMI	30.7 ± 5.6	29.7 ± 5.6	31.8 ± 5.4
Amputation duration (years)			8.7 ± 9.3
Classification			
Right transtibial			6
Left transtibial			8
Bilateral transtibial			3

Table 1. Participant characteristics. Values are (m ± sd).

Statistical analysis

A 2 (group) by 2 (walk test) by 3 (time point) factorial ANOVA was used to identify differences in VO_2 as well as in HR at rest, 1-min, and 2-min of each walking test. To evaluate steady state in the 6-min test, 2 (group) × 6 (time point) factorial ANOVAs with repeated measures on the second factor were used to explore differences in VO_2 and in HR at minutes 1 through 6. Differences in steps taken and for distance walked at 1 min and 2 min of each walking test, as well as between groups at minute 6, were analyzed with a 2 (group) by 2 (walk test) by 4 (time point) factorial ANOVA. Differences in cadence, gait speed, stance ratio, and stride length of each walking test were analyzed with a 2 (group) by 2 (walk test) factorial ANOVA. Bonferroni technique was applied when examining pairwise comparisons. Values are expressed as means (m) ± standard deviations (sd). Pearson's correlations were used to establish the relationship between the 2MWT and 6MWT, and regression analysis was used to assess the predictability of the 6MWT from the 2MWT. Alpha was set at 0.05 for all tests.

Results

VO_2 at rest, minute 1, and minute 2 of each walking test

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.014$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. No significant interaction was evident for group and walk ($F_{(2.37, 75.79)} = 2.21$, $p = 0.107$, $\eta_p^2 = 0.065$), however, there was a main effect of time ($F_{(2.37, 75.79)} = 136.6$, $p = 0.001$, $\eta_p^2 = 0.810$), such that VO_2 of iLLAs during the 2MWT increased from minute 1 (5.9 ± 1.8 ml/kg/min) to minute 2 (10.6 ± 3.2 ml/kg/min) ($p = 0.001$) as well as during the 6MWT from minute 1 (5.8 ± 2.1 ml/kg/min) to minute 2 (10.6 ± 2.9 ml/kg/min) ($p = 0.001$). These same trends occurred in the unimpaired group, with VO_2 significantly increasing during the 2MWT from minute 1 (5.2 ± 1.4 ml/kg/min) to minute 2 (11.9 ± 3.1 ml/kg/min) ($p = 0.001$), and during the 6MWT from minute 1 (5.2 ± 1.1 ml/kg/min) to minute 2 (12.2 ± 3.9 ml/kg/min) ($p = 0.001$), Fig. 1.

There were no significant differences in VO_2 between groups at rest ($p = 0.092$), at 1 min ($p = 0.240$), and at 2 min ($p = 0.265$) during the 2MWT. Non-significant findings were also evident for VO_2 during the 6MWT at rest ($p = 0.938$), at 1 min ($p = 0.312$), and at 2 min ($p = 0.190$), Fig. 1.

There was no significant difference in VO_2 of those with prothesis between walking tests at rest ($p = 1.0$), at 1 min ($p = 1.0$), and at 2 min ($p = 1.0$), or VO_2 of the unimpaired group at rest ($p = 0.704$), at 1 min ($p = 1.0$), and at 2 min ($p = 1.0$), Fig. 1.

HR at rest, minute 1, and minute 2 of each walking test

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.041$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. While there was no significant interaction for group and walk ($F_{(2.26, 63.28)} = 0.376$, $p = 0.714$, $\eta_p^2 = 0.013$), there was a main effect of time ($F_{(2.26, 63.28)} = 105.19$, $p = 0.001$, $\eta_p^2 = 0.796$). Following the same trends in VO_2, there was a significant difference in HR of iLLA during the 2MWT between minutes 1 (107.7 ± 15.9) and 2 (115.7 ± 22.1) ($p = 0.001$) as well as during the 6MWT between minutes 1 (105.3 ± 14.4) and 2 (112.8 ± 16.9) ($p = 0.001$). This occurred similarly in the unimpaired group, with HR significantly increasing during the 2MWT from minute 1 (97.5 ± 11.8) to minute 2 (105.9 ± 13.7) ($p = 0.002$), and during the 6MWT from minute 1 (97.9 ± 11.2) to minute 2 (107.2 ± 13.6) ($p = 0.001$), Fig. 2.

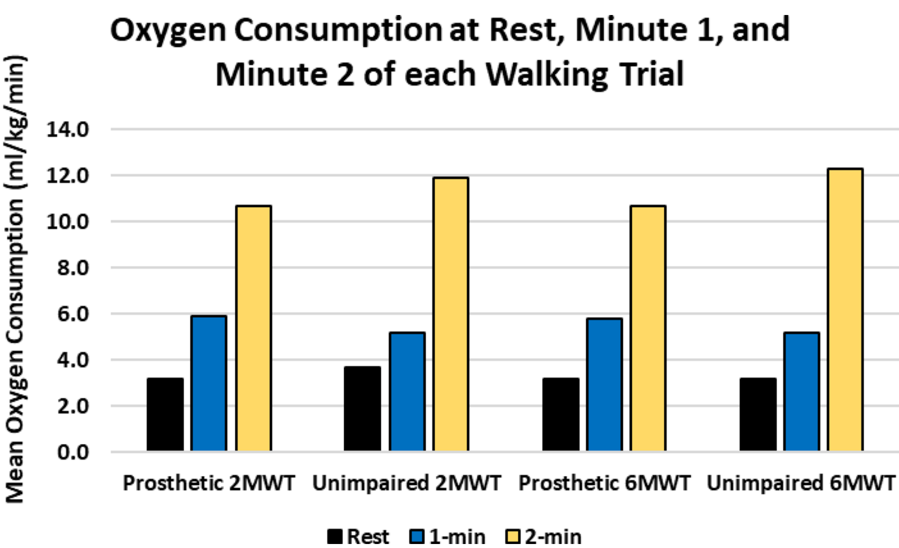

Fig. 1. While there were no significant differences in oxygen consumption (VO_2) between prosthesis users and unimpaired groups at any time point ($p > 0.05$), VO_2 increased significantly in both groups from rest, to minute 1, and to minute 2 ($p < 0.001$) during both walking tests. *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

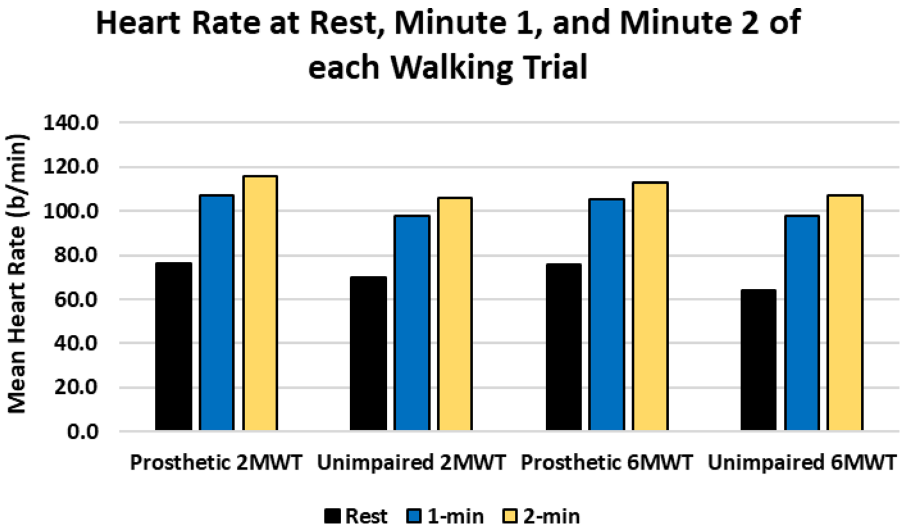

Fig. 2. While there were no significant differences in heart rate (HR) between prosthesis users and unimpaired groups at any time point ($p > 0.05$), HR increased significantly in both groups from rest, to minute 1, and to minute 2 ($p < 0.001$) during both walking tests. *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

There were no significant differences in HR between groups at rest ($p = 0.0107$), at 1 min ($p = 0.071$), and at 2 min ($p = 0.149$) during the 2MWT. Non-significant findings were also evident for HR during the 6MWT at rest ($p = 0.053$), at 1 min ($p = 0.127$), and at 2 min ($p = 0.330$), Fig. 2.

There was also no significant difference in HR of those with prosthesis between walking tests at rest ($p = 1.0$), at 1 min ($p = 1.0$), and at 2 min ($p = 1.0$), or in HR of the unimpaired group at rest ($p = 1.0$), at 1 min ($p = 1.0$), and at 2 min ($p = 1.0$), Fig. 2.

VO2 during minutes 1 through 6 of the 6MWT

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.007$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. There was a significant interaction for group and time ($F_{(1.67, 53.49)} = 4.30$, $p = 0.021$, $\eta_p^2 = 0.118$), where VO_2 in iLLA increased from minute 1 (5.8 ± 2.1 ml/kg/min) to minute 2 (10.6 ± 2.9 ml/kg/min) ($p = 0.001$), and from minute 2 to minute 3 (12.7 ± 3.3 ml/kg/min) ($p = 0.001$). This was also evident in the unimpaired group from minute 1 (5.2 ± 1.1 ml/kg/min) to minute 2 (12.2 ± 3.9 ml/kg/min) ($p = 0.001$), and from minute 2 to minute 3 (14.7 ± 3.7 ml/kg/min) ($p = 0.001$). The only other significant difference in time point was between minute 3 and minute 5 (15.8 ± 4.3 ml/kg/min) of the unimpaired, $p = 0.038$, Fig. 3.

There were no significant differences in VO_2 between groups at any of the time points ($p > 0.05$), Fig. 3.

HR during minutes 1 through 6 of the 6MWT

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.004$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. There was no significant interaction for group and time ($F_{(1.51, 45.50)} = 0.361$, $p = 0.640$, $\eta_p^2 = 0.012$), however, HR in iLLA increased from minute 1 (104.3 ± 13.9 b/min) to minute 2 (111.4 ± 16.3 b/min) ($p = 0.001$), from minute 2 to minute 3 (114.9 ± 17.7 b/min) ($p = 0.007$), and from minute 3 to minute 4 (117.2 ± 17.9 b/min) ($p = 0.002$). This same tend followed with the unimpaired group, with significant increases from minute 1 (97.9 ± 11.2 b/min) to minute 2 (107.2 ± 13.6 b/min) ($p = 0.001$), from minute 2 to minute 3 (110.3 ± 15.3 b/min) ($p = 0.032$), and from minute 3 to minute 4 (112.7 ± 16.6 b/min) ($p = 0.001$), Fig. 4.

There were no significant differences in HR between groups at any of the time points ($p > 0.05$), Fig. 4.

Differences in steps taken and distance walked

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.021$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. There was no significant interaction with steps taken between groups ($F_{(1.12, 34.98)} = 2.08$, $p = 0.156$, $\eta_p^2 = 0.063$). There were no significant differences between groups in steps taken at any of the time points during each walk test ($p > 0.05$), Fig. 5.

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2_{(14)} = 0.002$, $p = 0.001$, thus the Greenhouse–Geisser was used for the critical *F*. There was a significant interaction with distance between groups ($F_{(1.05, 33.65)} = 6.44$, $p = 0.015$, $\eta_p^2 = 0.168$). Those with prosthesis walked significantly less distance compared to the unimpaired group at minute 1 (81.3 ± 20.2 m and 94.2 ± 12.2 m, respectfully, $p = 0.030$) and 2 (164.9 ± 41.1 m and 190.7 ± 23.5 m, respectfully, $p = 0.031$) of the 2MWT, as well as minute 2 (159.8 ± 38.2 m and 192.3 ± 23.5 m, respectfully, $p = 0.005$) and minute 6 (480.0 ± 120.6 m and 574.9 ± 87.5 m, respectfully, $p = 0.013$) of the 6MWT, Fig. 6.

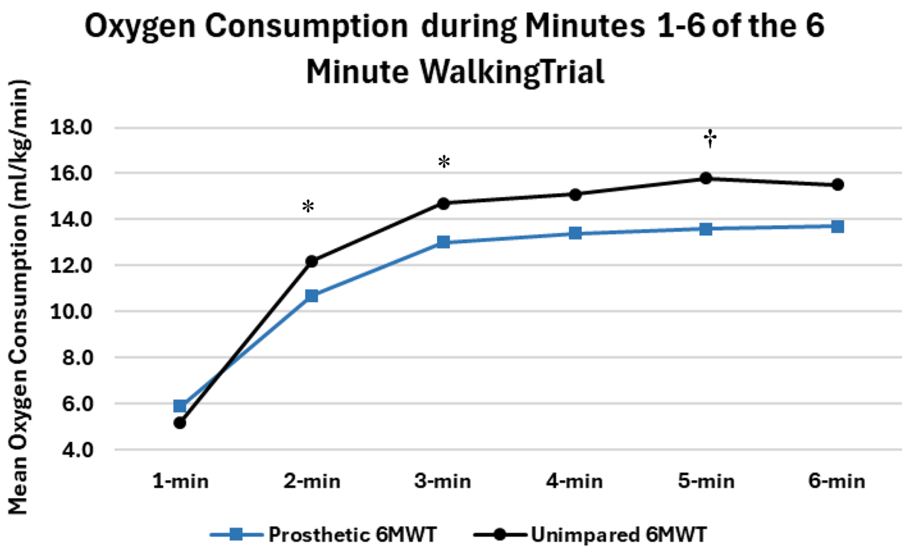

Fig. 3. There was a significant increase in VO_2 of both groups from minute 1 to minute 2, and from minute 2 to minute 3 (*$p > 0.05$). VO_2 in minute 5 was also significantly higher than minute 3, but only in the unimpaired group (†$p > 0.05$). No significant difference in VO_2 existed between groups at any time point ($p > 0.05$). *Note*: 6MWT: 6 minute walk test.

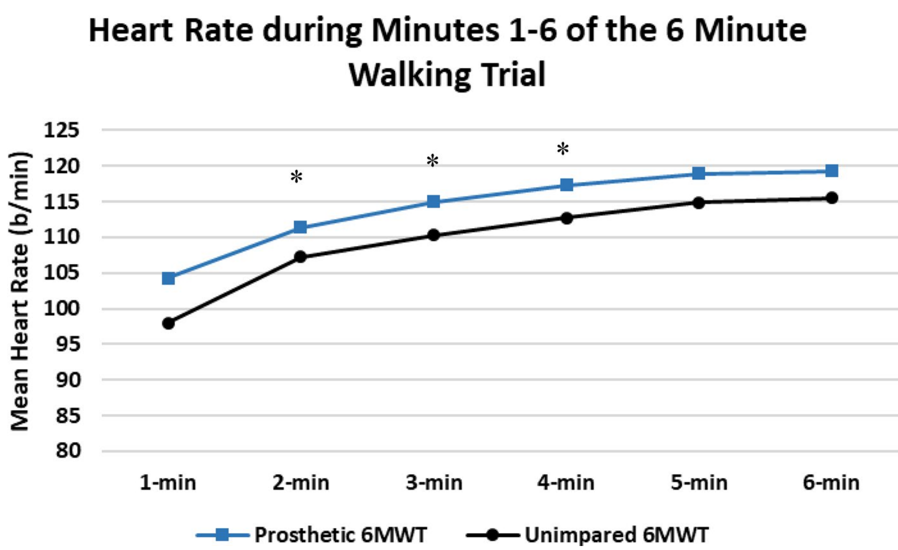

Fig. 4. There was a significant increase in HR of both groups from minute 1 to minute 2, from minute 2 to minute 3, and from minute 3 to minute 4 (*$p > 0.05$). No significant difference in HR existed between prosthesis users and unimpaired persons at any time point ($p > 0.05$). *Note*: 6MWT: 6 minute walk test.

There were no significant differences in distance walked at the 2 min time point between walking tests in those with amputations ($p = 0.319$) and in those without amputations ($p = 1.0$).

Differences in cadence and gait speed

There was no significant interaction with cadence between groups ($F_{(1, 32)} = 2.81$, $p = 0.103$, $\eta_p^2 = 0.081$), nor were there main effects for group ($p > 0.05$) or time ($p > 0.05$). Both groups had similar cadence during each test, Fig. 7.

There was also no significant interaction with gait speed between groups ($F_{(1, 32)} = 3.74$, $p = 0.062$, $\eta_p^2 = 0.105$); however, the unimpaired group walked significantly faster (1.50 ± 0.16 m/s) than iLLA (1.23 ± 0.31 m/s) during the 6MWT ($p = 0.005$), but not the 2MWT.No significant differences in gait speed were evident in unimpaired group between walking tests ($p = 0.068$), nor for iLLA ($p = 0.402$), Fig. 8.

Differences in stance ratio and stride length

There was no significant interaction with stance ratio between groups ($F_{(1, 32)} = 1.17$, $p = 0.286$, $\eta_p^2 = 0.036$), however, the unimpaired group had significantly lower stance ratio (59.0 ± 2.0% of ground contact time)

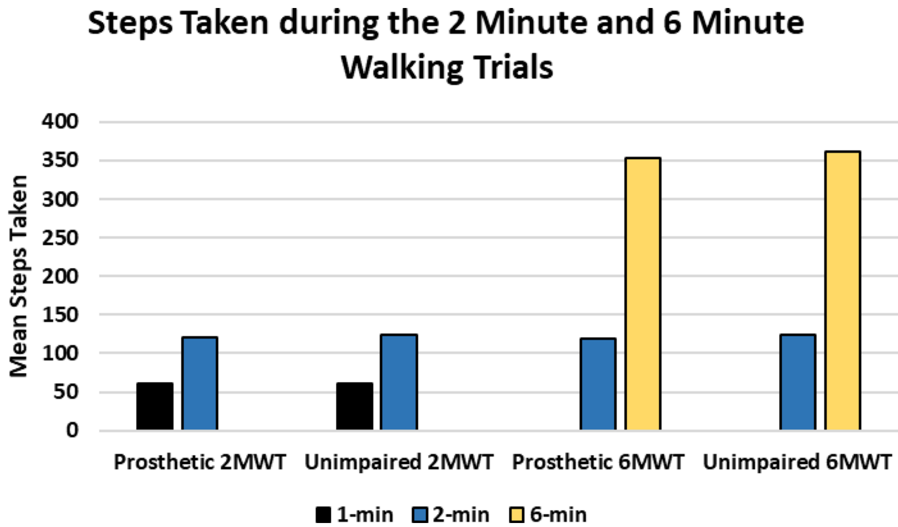

Fig. 5. Unimpaired and those with prosthesis took a similar number of steps at each time point during each walk test ($p > .05$). *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

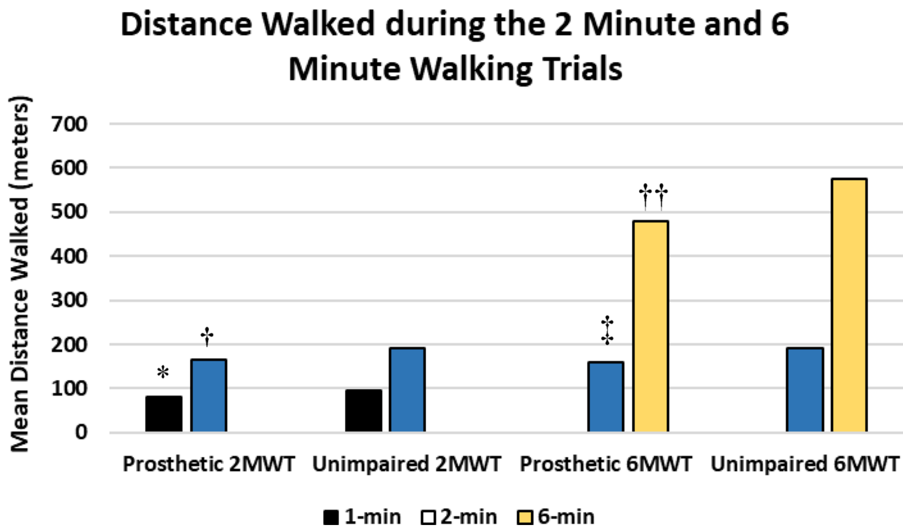

Fig. 6. Those with prosthesis walked significantly less distance than the unimpaired group at minute 1 (*$p = 0.030$) and 2 ($^{\dagger}p = 0.031$) of the two minute walk, as well as minute 2 ($^{\ddagger}p = 0.005$) and 6 ($^{\dagger\dagger}p = 0.013$) of the 6 min walk. *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

compared to iLLA (60.9 ± 2.1% of ground contact time) during the 2MWT ($p = 0.013$) as well as the 6MWT (59.0 ± 1.8 and 61.5 ± 2.2% of ground contact time, respectively, $p = 0.002$). There were no significant differences in stance ratio of the unimpaired group between walking tests ($p = 0.851$), nor for iLLA ($p = 0.094$), Fig. 9.

A significant interaction between group and stride length existed ($F_{(1, 32)} = 4.46$, $p = 0.042$, $\eta_p^2 = 0.123$) such that the unimpaired group had significantly longer strides (1.44 ± 0.11 m) than iLLA (1.26 ± 0.25 m) during the 2MWT ($p = 0.013$). This same trend was evident during the 6MWT (1.46 ± 0.11 m and 1.23 ± 0.26 m respectively, $p = 0.003$). While there were no significant differences in stride length of the unimpaired group between the two walking tests ($p = 0.355$), iLLA had significantly longer strides during the 2MWT compared to the 6MWT, $p = 0.048$ (Fig. 10).

While the relationship between the distance walked between the 2MWT and the 6MWT was strong and significant in unimpaired participants ($r_{(15)} = 0.76$, $p = 0.001$), it was much stronger in iLLAs ($r_{(15)} = 0.94$, $p = 0.001$) (Fig. 11). Simple regression analysis for unimpaired was significant ($p = 0.001$) with a resulting equation of predicted 6MWT distance = 21.6 + (2MWT distance * 2.9), with an SEE of 58.2 m and a mean absolute error of 35.7 ± 42.7 m. The simple regression equation for iLLAs was also significant ($p = 0.001$) and is as follows: predicted 6MWT distance = 27.0 + (2MWT distance * 2.7), with an SEE of 43.2 m and a mean absolute error of 33.4 ± 26.6 m.

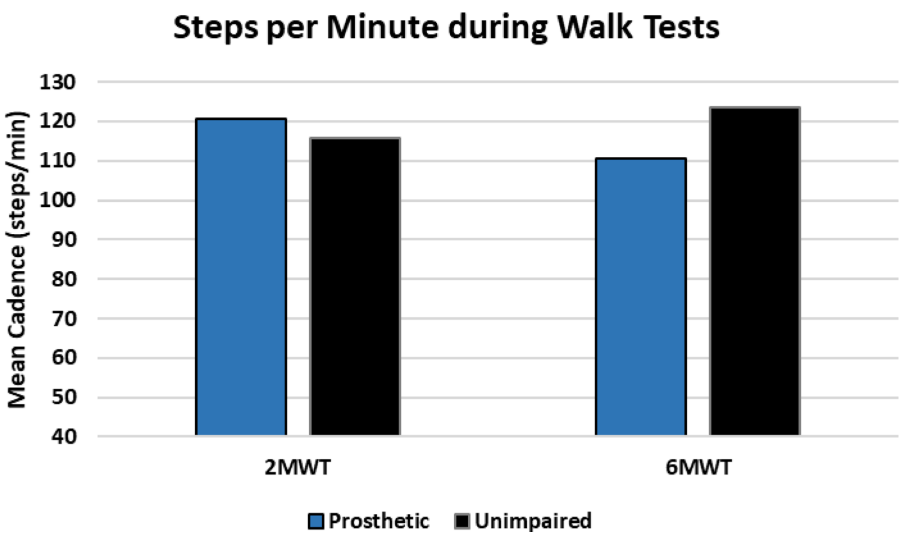

Fig. 7. There were no significant differences in cadence between groups and between tests ($p > 0.035$). *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

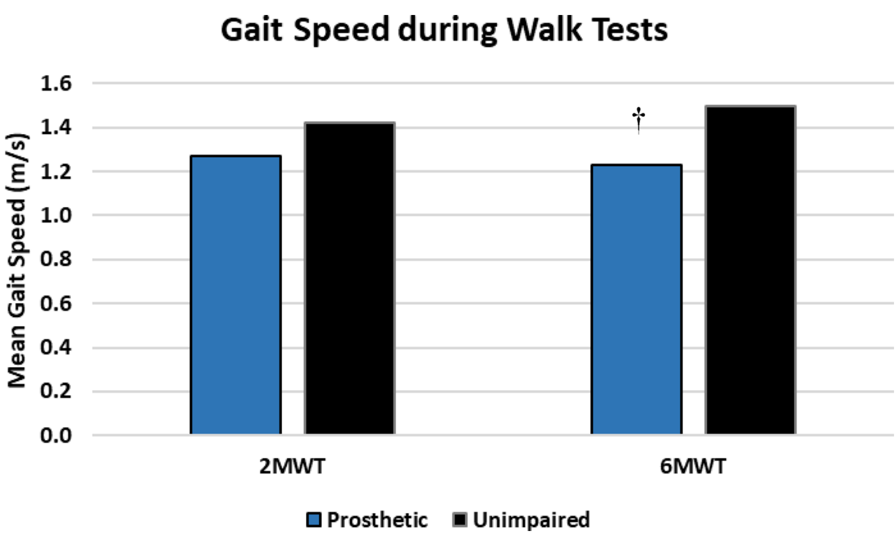

Fig. 8. Those with prosthesis walked significantly slower than the unimpaired group during the 6 min walk tests ($^{\dagger}p = 0.005$). *Note*: m/s: meters per second, 2MWT: 2 Minute Walk Test, 6MWT: 6 Minute Walk Test.

Discussion

This study compared metabolic and biomechanical dynamics of lower limb prosthesis users and unimpaired persons in two widely used walking outcome measures. Neither iLLAs users nor unimpaired persons achieved steady state oxygen uptake during the 2MWT nor during the first two minutes of the 6MWT. This was evident from significant differences in oxygen uptake during the first two minutes of both tests. There were no significant differences in oxygen uptake at any time point between iLLAs and unimpaired persons during the 2MWT. Based on these results, it is clear that 2MWT was not long enough for either group to have reached a metabolic steady state. Similarly, during the 6MWT both groups exhibited significant increases in VO_2 from minutes 1 to 2, but also minutes 2 to 3. Prosthesis users achieved steady state oxygen uptake after 3 min, however, unimpaired persons had significantly different VO_2 from minute 3 to 5. Although prosthesis users had reduced VO_2 during the 6MWT, these differences were not significant. Others have also observed no differences in VO_2 between iLLAs and unimpaired persons during overground walking[35,36]. Perhaps a higher exercise intensity or work load may have elicited a higher physiological demand resulting in differences in VO_2. This was the case when iLLAs and unimpaired controls carried a 32.7 kg external load, with iLLAs increasing their VO_2 compared to unimpaired controls[37].

Heart rate responses followed a similar trend to VO_2 data. Heart rates differed significantly between the first two minutes of the 2 and 6MWT for both iLLAs and unimpaired persons. Prosthesis users had significantly higher HR at minute 1 and 2 compared to unimpaired persons during the 2MWT. Although heart rate and

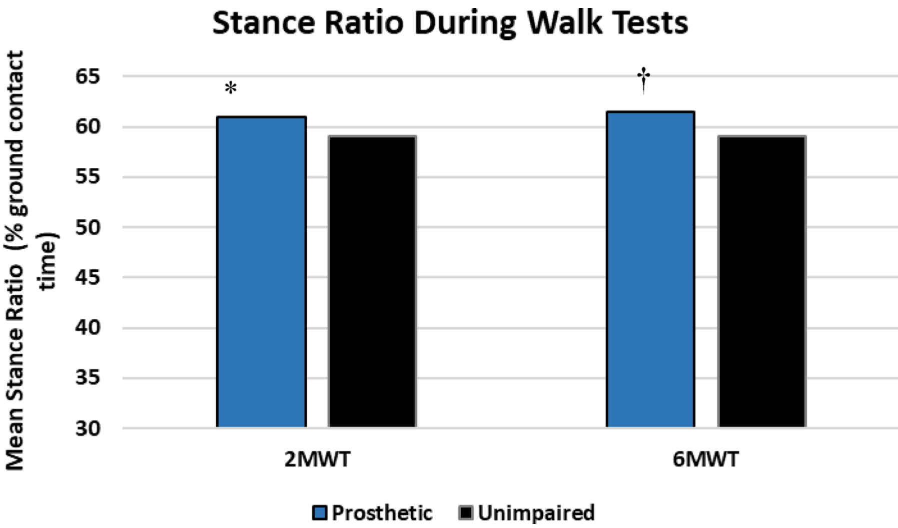

Fig. 9. Those with prosthesis had significantly greater stance ratio compared to the unimpaired group during the 2 min (*$p = 0.013$) and 6 min walk (†$p = 0.002$). *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

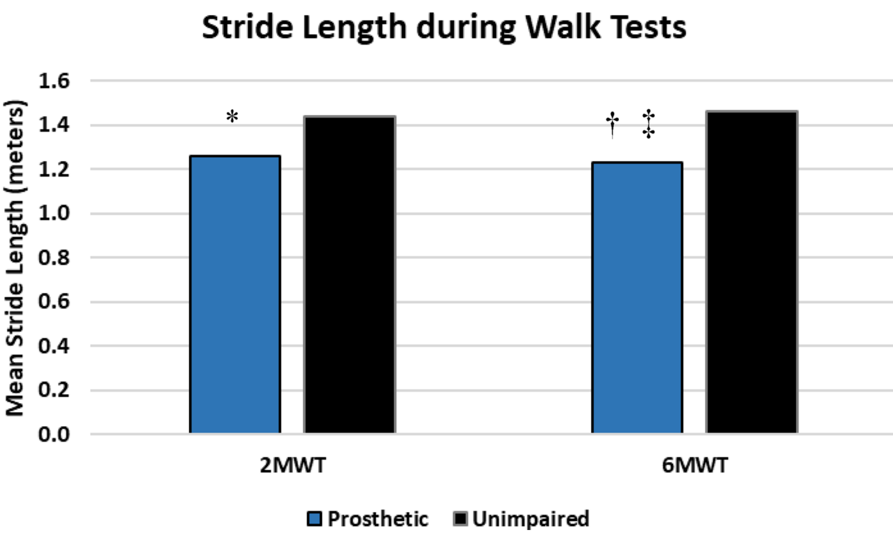

Fig. 10. Those wearing prosthesis had significantly shorter stride length compared to the unimpaired group during the 2 min (*$p = 0.013$) and 6 min walk (†$p = 0.003$). Furthermore, prosthesis wearers had significantly shorter stride length during the 6 min walk compared to the 2 min walk (‡$p = 0.048$). *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

VO_2 have a linear relationship[18], internal factors (i.e., fitness, mood) and external factors (i.e., environment, hydration) can influence HR response in a highly variable manner[38]. This may explain why although a plateau in VO_2 was observed for iLLAs midway through the 6MWT, heart rates continued to increase significantly even at minute 4. This trend was also observed in unimpaired persons. When comparing groups, although HR was higher in iLLAs, differences were not significant. Others have also observed increased heart rates in iLLAs compared to unimpaired during walking[36,39]. However, an individual's cardiovascular fitness level can improve with exercise training, which can improve the heart rate response to a given exercise[40,41]. The results of this study suggest that the 6MWT is long enough in duration for prosthesis users to achieve steady state VO_2 and HR, but that the 2MWT is not.

Differences in iLLAs biomechanics between walking tests help elucidate possible mechanisms influencing the observed physiological responses. Prosthesis users walked slower during the 6MWT (1.18 ± 0.031 m/s) than in the 2MWT (1.22 ± 0.32 m/s). These speeds are similar to that seen in a recent study by Younesian et al. who observed a speed of 1.13 m/s during the 6MWT and decreases in stance ratio across time. Stance ratio, however, did not differ between walking tests in our iLLA participants. Although, iLLA stride lengths were significantly lower (3 cm) during the 6MWT. Reducing stride length to increase the backward margins of stability (BW MoS)

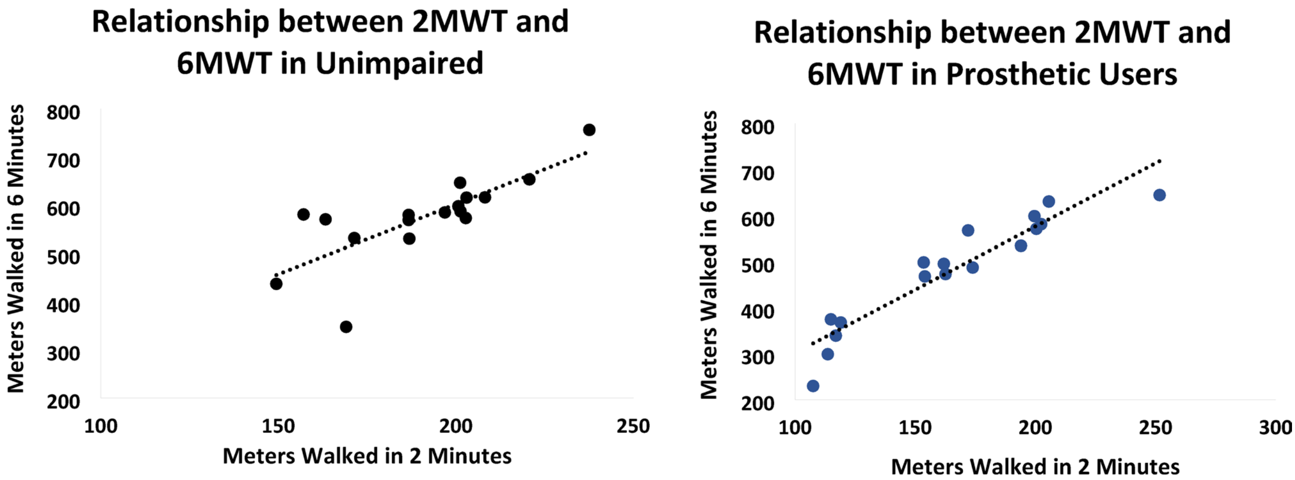

Fig. 11. The 2MWT is a strong predictor of 6MWT in unimpaired persons ($r_{(15)} = 0.76$, $p = 0.001$) and stronger in those using prostheses ($r_{(15)} = 0.94$, $p = 0.001$). *Note*: 2MWT: 2 minute walk test, 6MWT: 6 minute walk test.

is a gait strategy often used in persons with gait impairments[42,43]. Prosthesis users have reduced push-off power from their prosthesis and often employ stride and step adaptations for successful gait[44]. Thus, reducing gait speed and stride length during the 6MWT with concomitant plateaus in physiology suggest that iLLAs adjusted gait for successful performance of the 6MWT. Although unimpaired persons walked faster than iLLAs during both tests (0.20 and 0.32 m/s for 2MWT and 6MWT, respectively), their speed was 0.08 m/s faster during the 6MWT than in the 2MWT. Stride lengths were greater in unimpaired compared to iLLAs for both tests (18 and 23 cm for 2MWT and 6MWT, respectively), and although not significant, unimpaired stride lengths increased during the 6MWT compared to the 2MWT. Unimpaired cadence was 16.2 steps/min higher than iLLAs during the 6MWT but not 2MWT. Both groups optimized biomechanics for 6MWT completion. Prosthesis users reduced stride length and speed in a manner that may have spared energy expenditure whereas unimpaired persons appear to have done the opposite. As such, unimpaired persons performed better on the 2MWT and 6MWT covering greater distances than iLLAs.

Similar to prior scholarship, we found the 2MWT to be a strong predictor of 6MWT in iLLA[7]. This predictability was also observed for our unimpaired participant group. However, based off the results of this research it is clear that iLLAs employ markedly different gait strategies with altered physiology when completing the 6MWT. Thus, although test distances may be correlated, the 6MWT offers a direct challenge to a iLLAs users fitness and functional capacity. Thereby providing the prosthetist an outcome measure for evaluating their patient's adaptability to a prolonged arduous walking task. Moreover, in unimpaired older adults, the 2MWT has recently shown to have no correlation with maximal aerobic capacity[45]. However, the utility of the 2MWT is its reduced burden on the iLLAs and pragmatic administration. Furthermore, there may be contraindications to the 6MWT for those with underlying health issues. Thankfully, the clinician has the option of either of these performance based assessments to choose from[46].

This study is not without its limitations. Our heterogenic convenient sample recruited transtibial prosthesis users and excluded transfemoral prosthesis users. This may have influenced our findings as transfemoral prosthesis users require greater energy expenditure of walking compared to transtibial users[47]. Moreover, Gaunaurd et al., have observed significant differences across amputation levels in distance walked on timed walking tests (152.9 ± 43.0 m vs. 135.6 ± 43.0 m) ($p < 0.05$), for transtibial and transfemoral users respectively[3]. Future work should assess transfemoral prosthesis users performances during these tests. However, transtibial prosthesis users make up a majority of the iLLAs receiving prosthetic rehabilitation[48]. Moreover, exploring age related differences in our group of iLLAs was not performed. Others have discovered age related differences in spatiotemporal measures between 2 and 6MWT healthy adults[49]. As we did not repeat tests, we were unable to observe the possibility of a learning effect. This is an important consideration when administrating performance based outcome measures that should be pursued. Our sample was primarily K3 level users with one K2 participant. Exploring the physiological and biomechanical responses to timed walking tests in K2 level users is an important next step in this research.

Conclusion

The present study demonstrated marked physiological and biomechanical differences in prosthesis users between the 2MWT and 6MWT. Despite the pragmatic utility, ease of administration, and distance predictability of the 2MWT, our findings do not support its use as a surrogate assessment of the 6MWT in iLLAs. Although a case can be made for selecting either of these tests. The 2MWT is appropriate for assessing gait performance in iLLAs over a short duration, whereas the 6MWT assesses steady state gait performance and functional capacity. Notwithstanding contraindications or other barriers, we encourage clinicians to utilize the 6MWT as it permits a comprehensive assessment of prosthesis users abilities.

Data availability
The datasets generated during and/or analyzed during the current study are not publicly available due to university regulations but are available from the corresponding author on reasonable request.

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Author contributions

JDS and GG wrote the main manuscript text, and GG prepared all figures. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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