Carbohydrate and fat oxidation in persons with lower limb amputation during walking with different speeds

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Abstract

Background: Studies suggest that the energy expenditure (EE) of healthy persons (CON) during walking with the preferred walking speed (PWS) in steady-state conditions, is dominated by fat oxidation. Conversely, carbohydrate (CHO) and fat oxidation during walking is little investigated in transfemoral amputees (TFA).

Objective: Investigate CHO and fat oxidation, energy cost of walking (ECW) and percent utilization of maximal aerobic capacity (%\(\dot{V}O_2\)max) during walking.

Study design: Eight TFA and CON walked with their PWS and speeds 12.5 and 25% slower and faster than their PWS.

Methods: EE and fuel utilization was measured using a portable metabolic analyzer. Metabolic values are means±SE.

Results: For TFA (37.0±10.9 yrs.) and CON (39.0±12.3 yrs.), fat utilization at the PWS was 44.8±7.2 and 45.0±7.2% of the total EE, respectively. The PWS the TFA and CON was close to a metabolic crossover speed, which is the speed where CHO utilization increases steeply, and fat utilization decreases. When walking fast, at 90 m min\(^{-1}\) (PWS plus 25%), TFA utilized 70.7±5.6% of their \(\dot{V}O_2\)max while the CON utilized 30.9±4.5% (p<0.001) at the matching speed (CON PWS). At 90 m min\(^{-1}\), CHO utilization was 78±4.7 and 55.2±7.2% of the total EE for the TFA and CON, respectively (p<0.01). Compared to the CON, ECW was higher for the TFA at all speeds (all comparisons; p<0.001).

Conclusion: At the PWS, carbohydrate, not fat, dominates EE of both TFA and CON. For the TFA, consequences of fast walking is very high \(\dot{V}O_2\)max utilization and rate of CHO oxidation.

Word count: 250
Clinical relevance

Research on the relationships between physical effort and fuel partitioning during ambulation could provide important insights for exercise-rehabilitation programs for lower limb amputees (LLA). Regular endurance exercise will improve maximal aerobic capacity and enable LLA to walk faster, and at the same time expend less energy and improve fat utilization.

Word count: 50

Keywords: Metabolism, Fat utilization, Carbohydrate utilization
Background

Walking is the most common form of exercise and may for many people be the only break in an otherwise sedentary life. Following a lower limb amputation and the resulting walking disability, persons often adopt a very sedentary lifestyle which may, over time, further reduce aerobic power, physical fitness, and walking speed. During level walking, the rate of oxygen uptake relates to the walking speed, hence, measurements of the rate of oxygen uptake ($\dot{V}O_2$) during prosthetic walking is an important tool for assessing the energetic consequences of walking disabilities. Previous research has shown that there may exist an individual, optimal walking speed with regard to minimal energy expenditure and measurement of walking economy at this preferred walking speed (PWS) is frequently used as an indicator of overall gait performance of prosthetic walkers. It is well known that the PWS of persons with a lower limb amputation is slower compared to healthy age-matched individuals and the walking economy (oxygen uptake per meter traveled) at the PWS is also substantially higher for persons with a lower limb amputation. The higher walking economy following lower limb loss is related both to the level and etiology of amputation, but it is argued that this could in part, be caused by the fact that persons with lower limb amputation cannot reach their optimal (most economical) walking speed. Consequently, the present study aims to explore the impact of different walking speeds on the walking economy and energy expenditure of both healthy persons and person with lower limb loss.

Moreover, there are very few studies on the relation between walking speed and fuel partitioning during prosthetic ambulation, but previous studies on healthy persons have demonstrated that fat is the preferred energy substrate when walking with the PWS. The quantity of energy from carbohydrate (CHO) stores of the body is only 1% of that available in fat and the rate of CHO oxidation increases with increasing physical effort.
Consequently, it is suggested that conservation of CHO energy reserves, rather than walking economy per se, governs the selection of a preferred walking speed, and that central nervous system (CNS) selects a PWS that is supported mainly by fat oxidation. Except for one previous study, looking at fuel utilization at the PWS during treadmill walking, data on carbohydrate and fat oxidation during prosthetic walking is virtually absent from the literature.

Thus, there is a need to investigate how overground walking with different speeds affect carbohydrate and fat oxidation rates and walking economy of persons with a unilateral transfemoral amputation and healthy, age and sex-matched individuals. The main hypotheses are that persons with a transfemoral amputation (TFA) have a higher rate of CHO oxidation at similar relative speeds compared to healthy persons. In addition, we hypothesize that differences in walking speeds will have little effect on the walking economy, but that the physical effort, quantified as percent utilization of the maximal oxygen uptake (\(\dot{V}O_2\text{max}\)) will differ substantially between TFA and healthy persons.
Methods

Participants

Two groups of participants were recruited to this study. The participants of the transfemoral amputee (TFA) group were eight, non-smoking adults (50 % females) with unilateral transfemoral amputation for other reasons than vascular diseases and no-comorbidities. Causes of amputations were: trauma (n=1), cancer (n=5), congenital (n=1) and infection (n=1). The TFA participants had in average (± SD) used their prosthesis for 15.9 ± 13.9 years (range 3 to 39 years). Five persons had a microcontroller assisted knee joint, while three persons used hydraulic controlled knee joints, and all TFA participants used their prosthesis on a daily basis. The average weight of the prostheses was 3.80 ± 0.5 kg. The participants in the control (CON) group were eight healthy, non-smoking adults (50 % females) with no orthopedic problems and with similar weight, height, age as the TFA. Exclusion criteria for both groups was use of medication that could affect heart rate or energy expenditure (i.e. beta-blockers and thyroid hormone replacements). Daily walking distance of both the TFA and CON was assessed by a self-report form and inclusion criteria was that the participants were able to walk continuously for at least 500 meters. Written informed consent was obtained from all subjects and the study was approved by the Regional Committees for Medical and Health Research Ethics in Norway.

Study design

The participants were instructed to avoid exercise and alcohol 24 hours prior to testing and to abstain from coffee and tea on the day of testing. The TFA and CON reported to the laboratory in the morning, two hours after eating a low-fat breakfast (bread, jam, sliced ham, juice, low-fat milk) and were subsequently instrumented for collection of expired air. During all walk trials, the \( \dot{V}O_2 \) consumption and \( VCO_2 \) production was measured breath-by-breath
with a validated\textsuperscript{17} and portable metabolic analyzer (Metamax 3B, Cortex Biophysik, Germany). Heart rate was recorded beat-by-beat (Polar, Finland), interfaced with the metabolic analyzer. During trials, participants walked with their PWS and with speeds that were 25\% and 12.5\% lower and higher than their respective PWS. The sequence of walking speeds was determined for each individual by having an independent person randomly select between five closed envelopes, each containing a note specifying one of five specific walking speeds. Each walking trial (speed) lasted seven minutes and data reported on physiological measurements and metabolic calculations are average values over the last 2 min of each walking interval. Each walking trial was interspaced by rest intervals of two minutes where the participants sat quietly on a chair. The walking trials were performed around a 40 meter oblong indoor course, and the walking speed was monitored by a five meter long optical gait analysis system (OptoGait, Microgate, Bolzano-Bozen, Italy). Prior to walking trials, the PWS was determined by having the participants walk a stretch of 10 meters, with the speed measured by the OptoGait system during the last five meters. This sequence was repeated twice and averaged. During trials, walking speed was measured twice for each 40 meter round, and if necessary, verbal instructions such as “walk a little slower/walk a little faster/keep the pace,” were given to the participants in order to adjust their speed.

Furthermore, the maximal aerobic capacity (\(\dot{V}O_2\text{max}\)) of the participants was determined according to previous protocols\textsuperscript{18} on a separate occasion, one to two weeks earlier than the walking trials. In short, the participants walked on a treadmill (Woodway ELG 70, Woodway Waukesha, USA) with constant speed, but with progressively increasing inclinations until volitional fatigue. The VO\(_2\) measurements were considered maximal when the oxygen uptake did not increase >2 mL min\(^{-1}\) kg\(^{-1}\) (plateau in VO\(_2\)) despite increasing workload and with respiratory exchange ratio (RER) values > 1.05\textsuperscript{19}
Gas exchange and energy expenditure measurements

The oxygen analyzer was calibrated for barometric pressure and gas calibrated with a reference gas mixture of 16 % O₂ and 4 % CO₂. The calibration was then verified with measurements of ambient air, according to the manufacturer’s instructions. In addition, a volume calibration was performed using a standardized 3 L syringe (Hans Rudolph, Kansas, USA).

VO₂, VCO₂, lung ventilation, heart rate and respiratory exchange ratio (RER) values were continuously monitored during testing by telemetry in real-time to verify steady state conditions during walking trials. The RER is the ratio between the carbon dioxide production and the oxygen consumption, and all walking sessions were completed with RER values < 1.0. Carbohydrate and fat oxidation was calculated by indirect calorimetry using standard methods. Protein oxidation was assumed to be insignificant during these walking trials. The energy cost of walking i.e. the oxygen consumption per unit distance (ECW; mL·kg⁻¹·m⁻¹) was calculated by dividing the participants’ VO₂ consumption (mL·kg⁻¹·min⁻¹) by their respective walking speed (m·min⁻¹).

Statistics

Independent t-tests were used to compare the TFA and CON for physical characteristics. A two-way mixed ANOVA was used to test if oxygen uptake (VO₂ mL·min⁻¹·kg⁻¹), percent VO₂max utilization, carbohydrate and fat oxidation rates (cal·kg⁻¹·min⁻¹) and walking economy (VO₂ mL·kg⁻¹·m⁻¹) differed across walking speeds. Post hoc comparisons with Bonferroni corrections were conducted in case of a significant ANOVA. Specifically, the values at the PWS were compared to values at each of the other walking speeds. Data were tested for normality by the Shapiro-Wilk test. In those instances where the sphericity
assumption was violated, Greenhouse–Geisser adjustments of the P values were reported. The criterion level for significance was set at $p < 0.05$. The effect size was evaluated with $\eta^2$ (partial eta squared), where $0.01 < \eta^2 < 0.06$ constitutes a small effect, $0.06 < \eta^2 < 0.14$ constitutes a medium effect, and $\eta^2 > 0.14$ constitutes a large effect\textsuperscript{22}. Pearson’s correlation was used to investigate the relationship between the pre-determined walking speeds and the actual measured walking speeds of the TFA and CON group. IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyzes. Results are presented as means ± standard deviations (SD) or means and confidence intervals (CI).
Results

Physical characteristics of the participants

The mean ± SD age, height, weight and body mass index of the TFA and CON were 37.0 ± 10.9 and 39.0 ± 12.3 years, 175.5 ± 4.6 and 170.0 ± 7.4 cm, 73.6 ± 10.4 and 72.7 ± 14.2 kg, 23.8 ± 2.7 and 25.2 ± 3.3 kg/m², respectively. The weight of the TFA is including their prosthesis. There were no statistical differences in physical characteristics between the two groups. The maximal aerobic capacity ($\dot{V}O_2$max) of the TFA and CON were 30.6 ± 8.7 and 48.9 ± 14.4 mL·min⁻¹·kg⁻¹, ($p < 0.05$), respectively. Mean daily, self-reported walking distance was 2187 ± 923 and 2688 ± 834 meters for the TFA and CON ($p = 0.243$). All TFA participants reported they were able to walk at least 500 meters continuously.

Walking speed

All participants walked with their preferred walking speed (PWS) and speeds 12.5 and 25 % slower and faster than their respective PWS, thus all relative walking speeds were similar for the TFA and CON. In terms of actual walking speed in m·min⁻¹, mean PWS of the TFA and CON were 73.2 ± 11.1 and 91.3 ± 8.8 m·min⁻¹ (TFA vs. CON; $p < 0.001$). The range of walking speeds from the slowest to the fastest walking speeds (PWS minus 25 % to PWS plus 25 %) were 54.8 ± 9.7 – 90.4 ± 13.2 and 69.0 ± 6.3 – 114.4 ± 10.9 m·min⁻¹ for the TFA and CON, respectively (TFA vs. CON, all comparisons, $p < 0.001$). Actual walking speeds were monitored by the Optogait system (described in the methods section), and there was a close correlation between OptoGait measurements and the pre-determined (calculated) walking speeds. For the TFA group, correlation coefficients for the measured and calculated walking speeds of PWS minus 25 and 12.5 %, the PWS, and the PWS plus 12.5 and 25 % s, were: 0.997 ($p < 0.001$), 0.992 ($p < 0.001$), 0.994 ($p < 0.001$), 0.997 ($p < 0.001$) and 0.998 ($p < 0.001$), respectively. For the CON group the correlation coefficients for the same speeds were:
0.984 (p < 0.001), 0.989 (p < 0.001), 0.985 (p < 0.001), 0.993 (p < 0.001) and 0.997 (p < 0.001), respectively.

**Oxygen uptake (Table 1)**

The oxygen uptake (mL·min⁻¹·kg⁻¹) following walking with different speeds is shown in table 1. There was no significant interaction between group*time $F(4,56) = 0.572$, $p = 0.684$, $\eta^2 = 0.039$, but there was a significant main effect of time $F(4,56) = 89.537$, $p < 0.001$, $\eta^2 = 0.865$ upon oxygen uptake. There was no significant main effect of group on oxygen uptake $F(1,14) = 1.027$, $p = 0.328$, $\eta^2 = 0.068$, hence, the oxygen uptake for the TFA and CON was similar at all speeds. Mean group differences (confidence interval) for oxygen uptake was 1.403 (-1.566 – 4.371).

Pairwise post-hoc comparisons (for time) with Bonferroni corrections showed that for the TFA, the oxygen uptake at the PWS was significantly higher compared to PWS minus 25% ($p < 0.001$) and PWS minus 12.5% ($p < 0.05$) and lower compared to PWS plus 25% ($p < 0.001$). For the CON, the oxygen uptake at the PWS was significantly higher compared to PWS minus 25% ($p < 0.001$) and PWS minus 12.5% ($p < 0.05$) and lower compared to PWS plus 12.5% ($p < 0.05$) and PWS plus 25% ($p < 0.001$).

**Percent $\dot{V}O_2$max utilization (Table 1)**

There was no significant interaction between group*time $F(4,56) = 1.406$, $p = 0.244$, $\eta^2 = 0.091$, but there was a significant main effect of time $F(4,56) = 75.747$, $p < 0.001$, $\eta^2 = 0.844$ and group $F(1,14) = 14.259$, $p < 0.01$, $\eta^2 = 0.505$ upon $\dot{V}O_2$max. Mean group difference (confidence interval) for percent $\dot{V}O_2$max utilization was 22.936 (9.909 -35.964).
Pairwise post-hoc comparisons (for time) with Bonferroni corrections showed that for the TFA, the % $\dot{V}O_2$max utilization at the PWS was significantly higher compared to PWS minus 25% ($p < 0.001$) and PWS minus 12.5% ($p < 0.01$) and lower compared to PWS plus 25% ($p < 0.001$). For the CON, the % $\dot{V}O_2$max utilization at the PWS was significantly higher compared to PWS minus 25% ($p < 0.01$) and lower compared to PWS plus 25% ($p < 0.001$).

**ECW, energy cost of walking (Figure 1)**

There was no significant interaction between group*time $F(4,56) = 1.107$, $p = 0.362$, $\eta^2 = 0.073$, but there was a significant main effect of time $F(4,56) = 3.180$, $p < 0.05$, $\eta^2 = 0.185$ and group $F(1,14) = 29.873$, $p < 0.001$, $\eta^2 = 0.681$ upon the ECW.

Across the different walking speeds, the range of ECW values for the TFA and the CON were 0.213 – 0.226 and 0.146 – 0.174, respectively. Hence, since oxygen uptake and walking speed change more or less in parallel, the within-group ECW values show only small changes across the range of walking speeds. Pairwise post-hoc comparisons with Bonferroni corrections showed that the ECW of the CON was significantly lower compared to the TFA at the all speeds (all comparisons, $p < 0.001$). For both the TFA and CON, the ECW at the PWS was similar to the ECW at the other walking speeds.

**Carbohydrate and fat oxidation rates (Figure 2)**

For carbohydrate, there was no significant interaction between group*time $F(4,56) = 0.576$, $p = 0.681$, $\eta^2 = 0.039$, but there was a significant main effect of time upon carbohydrate oxidation rates $F(4,56) = 41.225$, $p < 0.001$, $\eta^2 = 0.746$. There was no significant main effect of group on carbohydrate oxidation rates $F(1,14) = 0.477$, $p = 0.328$, $\eta^2 = 0.037$. 
At similar relative walking speeds, the mean difference in carbohydrate oxidation rates between the TFA and CON was small, and varied between 1 and 10 cal kg\(^{-1}\) min\(^{-1}\). For both the TFA and CON, the carbohydrate oxidation rates at the PWS was significantly higher compared to PWS minus 25\% (both, \(p < 0.05\)) and lower compared to oxidation rates at PWS plus 25\% (\(p < 0.001\)).

For fat, there was no significant interaction between group*time \(F(4,56) = 0.858, p = 0.495, \eta^2 = 0.085\), no any significant main effect of time \(F(4,56) = 1.304, p = 0.280, \eta^2 = 0.085\) or group \(F(1,14) = 0.023, p = 0.881, \eta^2 = 0.002\) upon fat oxidation rates.

At similar relative walking speeds, the fat oxidation rates of the TFA and CON were quite similar and mean difference in fat oxidation rates were in the order of 2 - 7 cal kg\(^{-1}\) min\(^{-1}\). Fat oxidation rates are shown in figure 2.
Discussion

The present study compared walking economy, percent utilization of the \( \dot{V}O_2 \text{max} \) and fuel utilization of healthy individuals and persons with lower limb amputation across a wide range of walking speeds. Our data show that the energy cost of walking of both the TFA and CON is virtually similar over the range of walking speeds investigated (Fig 1). The explanation for this, is that as the walking speed changes, the oxygen uptake changes in parallel, thus the energy cost of walking remain stable for both groups. The TFA, however, have a significantly higher energy cost compared to the CON. As table 1 show, the oxygen uptakes of the TFA and CON are similar at similar relative walking speeds. Consequently, since the energy cost of walking is calculated as the oxygen uptake per meter travelled, the differences between lower limb amputees and healthy persons are chiefly the result of the much lower walking speed of the TFA. Based on this assumption, it is our opinion that the energy cost of walking do not provide much unique information about the effort of prosthetic ambulation that is useful in a clinical setting. We suggest that using calculations of percent utilization of the individual maximal aerobic capacity (\% \( \dot{V}O_2 \text{max} \)) is a better way of describing the physical effort of prosthetic gait than the energy cost of walking.

Our results also show that carbohydrate and fat oxidation rates are similar for the TFA and CON when walking with similar relative speeds in the fed state. Thus, in the fed state there is little support for the hypothesis that the central nervous system naturally select a walking speed that require little or no net carbohydrate depletion\(^\text{12}\). Generally, the present study show that in the fed state, the preferred walking speed of the TFA is a *metabolic cross-over speed*\(^\text{23}\) meaning that when the TFA walking speed exceeds the preferred walking speed, the carbohydrate oxidation rates increases more steeply and there is a concomitant reduction in the fat oxidation rates (Fig 2). A similar response is seen for the CON, and at the same relative speeds, the curves are virtually overlapping with the TFA curves. However, at the
preferred walking speed, the TFA and CON walk at an absolute speed of 73 and 91 m·min⁻¹, thus the metabolic cross-over speed of the CON is at a higher absolute speed compared to the TFA. Furthermore, while the oxygen consumption at the preferred walking speed is similar (Table 1), the TFA utilized about 55 % of their $\dot{V}O_2$max, while the CON used about 31 % of their $\dot{V}O_2$max. Thus, the physical effort of walking at the preferred walking speed (PWS) is substantially greater for the TFA compared to the CON. Thus, when healthy persons walk at a leisurely pace (i.e. ~90 m·min⁻¹), persons with a lower limb amputation struggle to keep the same pace, because in order to do so, they will need to increase their speed by about 25 %. The consequences of increasing the speed to 90 m·min⁻¹ (PWS plus 25%), is that the TFA utilized 71 % of their $\dot{V}O_2$max (Table 1) and close to 80 % of the total energy expenditure was provided by carbohydrate oxidation (Fig 2). In contrast, at the same absolute speed, the CON utilized only 31 % of their $\dot{V}O_2$max and about 55 % of their energy expenditure was provided by carbohydrate oxidation. At 90 m·min⁻¹, the oxygen uptakes are similar for the TFA and CON and the energy cost of walking for both groups are unchanged compared to the preferred walking speed. This underscores that the energy cost of walking is not a good indicator of the physical effort of prosthetic ambulation.

As can be observed in figure 2, there is a great reliance on carbohydrate oxidation during fast walking and it is well established that endurance capacity and carbohydrate availability is highly interrelated, hence the size of the carbohydrate stores in the body may be of special importance for persons with a lower limb amputation. Pertaining to this, persons with a transfemoral amputation evidently have less lower extremity muscle mass than healthy persons and many are untrained and have low aerobic capacity. The carbohydrate (glycogen) stores in skeletal muscles is limited by the quantity of muscle mass and the intrinsic glycogen storage capacity of muscle tissue (80-150 mmol·kg⁻¹ wet weight). However, skeletal muscle of untrained individuals may store only 50 % of the capacity of
trained skeletal muscle\textsuperscript{27-29}. Thus, it is plausible that an untrained muscle mass and
corresponding low intramuscular carbohydrate stores may considerably limit the endurance
capacity and walking range of persons with lower limb amputation.

Furthermore, it is important to note that the rates of fat oxidation in the study of Willis et al.\textsuperscript{12},
is higher than the fat oxidation rates in the present study. In the above study, fat accounted for
about 65\% of the total energy expenditure during walking with the preferred walking speed,
and in a similar study of persons with post-stroke hemiparesis, fat supplied 58\% of the total
energy expenditure when walking with the preferred walking speed\textsuperscript{30}. In the present study, fat
utilization was about 45\% of the total energy expenditure for TFA and CON when walking
with the PWS, hence fat combustion was substantially lower than in the studies of Willis et al.
\textsuperscript{12} and Ganley et al.\textsuperscript{30}.

The reason for these discrepancies, may be related to the fact that the participants in the above
studies were tested following an overnight fast, and it is expected that fat oxidation rates are
quite high following prolonged fasting (> 6 hours)\textsuperscript{31}. On the other hand, to investigate fuel
oxidation during normal living, it is important to study the energy metabolism also in the non-
fasted state, as in the present study. The following example illustrates the impact of prior
feeding on fuel partitioning: In the Willis et al.\textsuperscript{12} study, the total energy expenditure of
healthy persons during treadmill walking with the treadmill preferred walking speed, was 63
cal min\textsuperscript{-1} kg\textsuperscript{-1}, and carbohydrate and fat utilization accounted for 33\% and 67\% of the total
energy expenditure, respectively. Oxygen uptake was 13.2 mL min\textsuperscript{-1} kg\textsuperscript{-1}, and heart rate was
95 beats min\textsuperscript{-1}. In a previous study in our lab\textsuperscript{32}, using the same participants as in the present
study, we observed that during treadmill walking at the preferred walking speed, CON mean
oxygen uptake, heart rate and total energy expenditure was 13.4 mL min\textsuperscript{-1} kg\textsuperscript{-1}, 92 beats min\textsuperscript{-1}
and 68 cal min\textsuperscript{-1} kg\textsuperscript{-1}, i.e. similar to the Willis study\textsuperscript{12}. However, carbohydrate and fat
oxidation was 64\% and 36\% of the total energy expenditure, respectively and consequently,
the fuel partitioning in the fed state (present study) was reversed with regard to the fasted state. This is, however, not unexpected as feeding suppresses fat oxidation during physical activity. Thus, to enable persons with a lower limb amputation to adopt a faster walking speed and at the same time expend less energy, it is of importance that these persons perform aerobic endurance exercise. Regular endurance exercise will over time improve the maximal aerobic capacity ($\dot{V}O_2\text{max}$) and switch substrate utilization towards fat oxidation. This may result in a clinically relevant reduction in the relative oxygen uptake during prosthetic ambulation and a more sustainable substrate utilization. Consequently, this may possibly translate into functional improvements in walking speed, walking endurance and a reduction in the perception of the physical effort. The interrelationship between improved physical work capacity and gait performance is little investigated in lower limb amputees, but Wezenberg et al. have developed a predictive quantitative model that show that even small increases in aerobic capacity can result in substantial improvements in walking ability of older adults with a lower limb amputation.

**Conclusion**

In the fed state, the preferred walking speed of both the TFA and CON is close to a metabolic cross-over walking speed above which carbohydrate oxidation rates increases steeply. Thus, when the TFA walking speed exceeds the preferred walking speed, there is an increasing reliance on carbohydrate oxidation and a concomitant reduction in the fat oxidation rates. The relative oxygen uptake ($VO_2$, ml min$^{-1}$ kg$^{-1}$) did not differ between groups across the range of walking speeds. Within each group, the energy cost of walking did not vary across walking speeds. In contrast, the $\%\dot{V}O_2\text{max}$ differed significantly across walking speeds for both groups. The $\%\dot{V}O_2\text{max}$ may be a better indicator of the physical effort of prosthetic ambulation than the energy cost of walking.
Possible limitations

The TFA in the present study used different types of knee joints (hydraulic or microprocessor controlled) and one may speculate if this in any way could affect measures of energy cost of walking or oxygen uptake values. There is no indication in our data that differences in knee joint construction affect these measurements. Nonetheless, it may be prudent to conduct further studies on this matter with a larger number of participants to investigate the relations with walking speed and fuel selection during prosthetic walking.

WORD COUNT: 4 000
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Declaration of Conflict of interests
The authors declare that there is no conflict of interest.

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Preparation of manuscript
All authors contributed equally in the preparation of this manuscript.
References


Figure 1. Energy cost of walking (ECW) of the TFA and CON during walking with similar relative speeds.

Legend figure 1. Values are means ± SD. *** p < 0.001, TFA compared to CON.
Figure 2. Fuel oxidation rates of the TFA and CON during walking on the floor with different speeds.

Legend Figure 2

Values are means ± SD. *** p < 0.001, CHO oxidation rates at the PWS of the TFA compared to other speeds. ### p < 0.001, CHO oxidation rates at the PWS of the CON compared to other speeds.
**Table 1.** Oxygen uptake and percent utilization of $\dot{V}O_2$max at different walking speeds

<table>
<thead>
<tr>
<th>Relative Speeds</th>
<th>Group</th>
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<th>Oxygen uptake, %$\dot{V}O_2$max</th>
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<td>PWS</td>
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<td>PWS</td>
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<td>CON</td>
<td>20.0‡‡‡</td>
<td>17.4 – 22.6</td>
</tr>
<tr>
<td></td>
<td>TFA</td>
<td>20.6‡‡‡</td>
<td>17.9 – 23.2</td>
</tr>
</tbody>
</table>

**Legend Table 1.**

- PWS = preferred walking speed, CI = confidence interval. *p < 0.05, **p < 0.01, ***p < 0.001; PWS compared to lower speeds.
- #p < 0.05, ###p < 0.001; PWS compared to faster speeds.