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

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7 **Terje Gjøvaag<sup>1</sup>, Peyman Mirtaheri<sup>2</sup>, Inger Marie Starholm<sup>1</sup>**

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9 <sup>1</sup>Oslo and Akershus University of Applied Sciences, Institute of Occupational Therapy,  
10 Prosthetics and Orthotics, Oslo Norway.

11 <sup>2</sup>Oslo and Akershus University of Applied Sciences, Institute of Mechanical, Electronics and  
12 Chemical Engineering, Oslo, Norway

13

14 **Corresponding author:**

15 Terje F. Gjøvaag

16 Institute of Occupational Therapy, Prosthetics and Orthotics

17 Oslo and Akershus University of Applied Sciences

18 POB 4, St. Olavs plass, N-0130 Oslo, Norway

19 Email: [terje.gjovaag@hioa.no](mailto:terje.gjovaag@hioa.no)

20 Telephone: +47 67236267

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## 1 **Abstract**

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

6 *Objective:* Investigate CHO and fat oxidation, energy cost of walking (ECW) and percent  
7 utilization of maximal aerobic capacity ( $\dot{V}O_{2\max}$ ) during walking.

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

10 *Methods:* EE and fuel utilization was measured using a portable metabolic analyzer.

11 Metabolic values are means $\pm$ SE.

12 *Results:* For TFA (37.0 $\pm$ 10.9 yrs.) and CON (39.0 $\pm$ 12.3 yrs.), fat utilization at the PWS was  
13 44.8 $\pm$ 7.2 and 45.0 $\pm$ 7.2% of the total EE, respectively. The PWS the TFA and CON was close  
14 to a *metabolic crossover speed*, which is the speed where CHO utilization increases steeply,  
15 and fat utilization decreases. When walking fast, at 90 m min<sup>-1</sup> (PWS plus 25%), TFA utilized  
16 70.7 $\pm$ 5.6% of their  $\dot{V}O_{2\max}$  while the CON utilized 30.9 $\pm$ 4.5% (p<0.001) at the matching  
17 speed (CON PWS). At 90 m min<sup>-1</sup>, CHO utilization was 78 $\pm$ 4.7 and 55.2 $\pm$ 7.2% of the total  
18 EE for the TFA and CON, respectively (p<0.01). Compared to the CON, ECW was higher for  
19 the TFA at all speeds (all comparisons; p<0.001).

20 *Conclusion:* At the PWS, carbohydrate, not fat, dominates EE of both TFA and CON. For the  
21 TFA, consequences of fast walking is very high  $\dot{V}O_{2\max}$  utilization and rate of CHO  
22 oxidation.

23

24 **Word count:** 250

25

1 **Clinical relevance**

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

6 **Word count:** 50

7 **Keywords:** Metabolism, Fat utilization, Carbohydrate utilization

8

## 1 **Background**

2 Walking is the most common form of exercise and may for many people be the only break in  
3 an otherwise sedentary life<sup>1</sup>. Following a lower limb amputation and the resulting walking  
4 disability, persons often adopt a very sedentary lifestyle<sup>2</sup> which may, over time, further reduce  
5 aerobic power, physical fitness, and walking speed. During level walking, the rate of oxygen  
6 uptake relates to the walking speed<sup>3</sup>, hence, measurements of the rate of oxygen uptake ( $\dot{V}O_2$ )  
7 during prosthetic walking is an important tool for assessing the energetic consequences of  
8 walking disabilities. Previous research has shown that there may exist an individual, optimal  
9 walking speed with regard to minimal energy expenditure<sup>3-5</sup> and measurement of walking  
10 economy at this preferred walking speed (PWS) is frequently used as an indicator of overall  
11 gait performance of prosthetic walkers.<sup>6, 7</sup> It is well known that the PWS of persons with a  
12 lower limb amputation is slower compared to healthy age-matched individuals<sup>8,9</sup> and the  
13 walking economy (oxygen uptake per meter traveled) at the PWS is also substantially higher  
14 for persons with a lower limb amputation<sup>10</sup>. The higher walking economy following lower  
15 limb loss is related both to the level<sup>6</sup> and etiology of amputation<sup>10</sup>, but it is argued that this  
16 could in part, be caused by the fact that persons with lower limb amputation cannot reach their  
17 optimal (most economical) walking speed<sup>11</sup>. Consequently, the present study aims to explore  
18 the impact of different walking speeds on the walking economy and energy expenditure of  
19 both healthy persons and person with lower limb loss.

20

21 Moreover, there are very few studies on the relation between walking speed and fuel  
22 partitioning during prosthetic ambulation, but previous studies on healthy persons have  
23 demonstrated that fat is the preferred energy substrate when walking with the PWS<sup>12</sup>. The  
24 quantity of energy from carbohydrate (CHO) stores of the body is only 1 % of that available  
25 in fat<sup>13</sup> and the rate of CHO oxidation increases with increasing physical effort.<sup>14, 15</sup>

1 Consequently, it is suggested that conservation of CHO energy reserves, rather than walking  
2 economy *per se*, governs the selection of a preferred walking speed, and that central nervous  
3 system (CNS) selects a PWS that is supported mainly by fat oxidation<sup>12</sup>. Except for one  
4 previous study, looking at fuel utilization at the PWS during treadmill walking<sup>16</sup>, data on  
5 carbohydrate and fat oxidation during prosthetic walking is virtually absent from the  
6 literature.

7 Thus, there is a need to investigate how overground walking with different speeds affect  
8 carbohydrate and fat oxidation rates and walking economy of persons with a unilateral  
9 transfemoral amputation and healthy, age and sex-matched individuals.

10 The main hypotheses are that persons with a transfemoral amputation (TFA) have a higher  
11 rate of CHO oxidation at similar relative speeds compared to healthy persons. In addition, we  
12 hypothesize that differences in walking speeds will have little effect on the walking economy,  
13 but that the physical effort, quantified as percent utilization of the maximal oxygen uptake (%  
14  $\dot{V}O_2\text{max}$ ) will differ substantially between TFA and healthy persons.

## 1 **Methods**

### 2 *Participants*

3 Two groups of participants were recruited to this study. The participants of the transfemoral  
4 amputee (TFA) group were eight, non-smoking adults (50 % females) with unilateral  
5 transfemoral amputation for other reasons than vascular diseases and no-comorbidities.  
6 Causes of amputations were: trauma (n=1), cancer (n=5), congenital (n=1) and infection  
7 (n=1). The TFA participants had in average ( $\pm$  SD) used their prosthesis for  $15.9 \pm 13.9$  years  
8 (range 3 to 39 years). Five persons had a microcontroller assisted knee joint, while three  
9 persons used hydraulic controlled knee joints, and all TFA participants used their prosthesis  
10 on a daily basis. The average weight of the prostheses was  $3.80 \pm 0.5$  kg.

11 The participants in the control (CON) group were eight healthy, non-smoking adults (50 %  
12 females) with no orthopedic problems and with similar weight, height, age as the TFA.

13 Exclusion criteria for both groups was use of medication that could affect heart rate or energy  
14 expenditure (i.e. beta-blockers and thyroid hormone replacements). Daily walking distance of  
15 both the TFA and CON was assessed by a self-report form and inclusion criteria was that the  
16 participants were able to walk continuously for at least 500 meters. Written informed consent  
17 was obtained from all subjects and the study was approved by the Regional Committees for  
18 Medical and Health Research Ethics in Norway.

19

### 20 *Study design*

21 The participants were instructed to avoid exercise and alcohol 24 hours prior to testing and to  
22 abstain from coffee and tea on the day of testing. The TFA and CON reported to the  
23 laboratory in the morning, two hours after eating a low-fat breakfast (bread, jam, sliced ham,  
24 juice, low-fat milk) and were subsequently instrumented for collection of expired air. During  
25 all walk trials, the  $\dot{V}O_2$  consumption and  $VCO_2$  production was measured breath-by-breath

1 with a validated<sup>17</sup> and portable metabolic analyzer (Metamax 3B, Cortex Biophysik,  
2 Germany). Heart rate was recorded beat-by-beat (Polar, Finland), interfaced with the  
3 metabolic analyzer. During trials, participants walked with their PWS and with speeds that  
4 were 25 % and 12.5 % lower and higher than their respective PWS. The sequence of walking  
5 speeds was determined for each individual by having an independent person randomly select  
6 between five closed envelopes, each containing a note specifying one of five specific walking  
7 speeds. Each walking trial (speed) lasted seven minutes and data reported on physiological  
8 measurements and metabolic calculations are average values over the last 2 min of each  
9 walking interval. Each walking trial was interspaced by rest intervals of two minutes where  
10 the participants sat quietly on a chair. The walking trials were performed around a 40 meter  
11 oblong indoor course, and the walking speed was monitored by a five meter long optical gait  
12 analysis system (OptoGait, Microgate, Bolzano-Bozen, Italy). Prior to walking trials, the  
13 PWS was determined by having the participants walk a stretch of 10 meters, with the speed  
14 measured by the OptoGait system during the last five meters. This sequence was repeated  
15 twice and averaged. During trials, walking speed was measured twice for each 40 meter  
16 round, and if necessary, verbal instructions such as “walk a little slower/walk a little  
17 faster/keep the pace,” were given to the participants in order to adjust their speed.

18 Furthermore, the maximal aerobic capacity ( $\dot{V}O_2\text{max}$ ) of the participants was determined  
19 according to previous protocols<sup>18</sup> on a separate occasion, one to two weeks earlier than the  
20 walking trials. In short, the participants walked on a treadmill (Woodway ELG 70, Woodway  
21 Waukesha, USA) with constant speed, but with progressively increasing inclinations until  
22 volitional fatigue. The  $VO_2$  measurements were considered maximal when the oxygen uptake  
23 did not increase  $>2 \text{ mL min}^{-1} \text{ kg}^{-1}$  (plateau in  $VO_2$ ) despite increasing workload and with  
24 respiratory exchange ratio (RER) values  $> 1.05$ <sup>19</sup>

25

## 1 *Gas exchange and energy expenditure measurements*

2 The oxygen analyzer was calibrated for barometric pressure and gas calibrated with a  
3 reference gas mixture of 16 % O<sub>2</sub> and 4 % CO<sub>2</sub>. The calibration was then verified with  
4 measurements of ambient air, according to the manufacturer's instructions. In addition, a  
5 volume calibration was performed using a standardized 3 L syringe (Hans Rudolph, Kansas,  
6 USA).

7 VO<sub>2</sub>, VCO<sub>2</sub>, lung ventilation, heart rate and respiratory exchange ratio (RER) values were  
8 continuously monitored during testing by telemetry in real-time to verify steady state  
9 conditions during walking trials. The RER is the ratio between the carbon dioxide production  
10 and the oxygen consumption, and all walking sessions were completed with RER values <  
11 1.0. Carbohydrate and fat oxidation was calculated by indirect calorimetry using standard  
12 methods<sup>20</sup>. Protein oxidation was assumed to be insignificant during these walking trials<sup>21</sup>.  
13 The energy cost of walking i.e. the oxygen consumption per unit distance (ECW; mL·kg<sup>-1</sup>·  
14 m<sup>-1</sup>) was calculated by dividing the participants  $\dot{V}O_2$  consumption (mL·kg<sup>-1</sup>·min<sup>-1</sup>) by their  
15 respective walking speed (m·min<sup>-1</sup>).

16

## 17 *Statistics*

18 Independent t-tests were used to compare the TFA and CON for physical characteristics.

19 A two-way mixed ANOVA was used to test if oxygen uptake ( $\dot{V}O_2$  mL min<sup>-1</sup> kg<sup>-1</sup>), percent  
20  $\dot{V}O_{2\max}$  utilization, carbohydrate and fat oxidation rates (cal kg<sup>-1</sup> min<sup>-1</sup>) and walking  
21 economy (VO<sub>2</sub> mL·kg<sup>-1</sup>·m<sup>-1</sup>) differed across walking speeds. Post hoc comparisons with  
22 Bonferroni corrections were conducted in case of a significant ANOVA. Specifically, the  
23 values at the PWS were compared to values at each of the other walking speeds. Data were  
24 tested for normality by the Shapiro-Wilk test. In those instances where the sphericity



1 assumption was violated, Greenhouse–Geisser adjustments of the  $P$  values were reported. The  
2 criterion level for significance was set at  $p < 0.05$ . The effect size was evaluated with  $\eta^2$   
3 (partial eta squared), where  $0.01 < \eta^2 < 0.06$  constitutes a small effect,  $0.06 < \eta^2 < 0.14$   
4 constitutes a medium effect, and  $\eta^2 > 0.14$  constitutes a large effect<sup>22</sup>. Pearson's correlation  
5 was used to investigate the relationship between the pre-determined walking speeds and the  
6 actual measured walking speeds of the TFA and CON group. IBM SPSS Statistics for  
7 Windows, version 24.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyzes.  
8 Results are presented as means  $\pm$  standard deviations (SD) or means and confidence intervals  
9 (CI).

10

11

## 1 **Results**

### 2 *Physical characteristics of the participants*

3 The mean  $\pm$  SD age, height, weight and body mass index of the TFA and CON were  $37.0 \pm$   
4  $10.9$  and  $39.0 \pm 12.3$  years,  $175.5 \pm 4.6$  and  $170.0 \pm 7.4$  cm,  $73.6 \pm 10.4$  and  $72.7 \pm 14.2$  kg,  
5  $23.8 \pm 2.7$  and  $25.2 \pm 3.3$  kg/m<sup>2</sup>, respectively. The weight of the TFA is including their  
6 prosthesis. There were no statistical differences in physical characteristics between the two  
7 groups. The maximal aerobic capacity ( $\dot{V}O_2\text{max}$ ) of the TFA and CON were  $30.6 \pm 8.7$  and  
8  $48.9 \pm 14.4$  mL $\cdot$ min<sup>-1</sup> $\cdot$ kg<sup>-1</sup>, ( $p < 0.05$ ), respectively. Mean daily, self-reported walking  
9 distance was  $2187 \pm 923$  and  $2688 \pm 834$  meters for the TFA and CON ( $p = 0.243$ ). All TFA  
10 participants reported they were able to walk at least 500 meters continuously.

### 11 *Walking speed*

12 All participants walked with their preferred walking speed (PWS) and speeds 12.5 and 25 %  
13 slower and faster than their respective PWS, thus all *relative* walking speeds were similar for  
14 the TFA and CON. In terms of actual walking speed in m $\cdot$ min<sup>-1</sup>, mean PWS of the TFA and  
15 CON were  $73.2 \pm 11.1$  and  $91.3 \pm 8.8$  m $\cdot$ min<sup>-1</sup> (TFA vs. CON;  $p < 0.001$ ). The range of  
16 walking speeds from the slowest to the fastest walking speeds (PWS minus 25 % to PWS plus  
17 25 %) were  $54.8 \pm 9.7 - 90.4 \pm 13.2$  and  $69.0 \pm 6.3 - 114.4 \pm 10.9$  m $\cdot$ min<sup>-1</sup> for the TFA and  
18 CON, respectively (TFA vs. CON, all comparisons,  $p < 0.001$ ). Actual walking speeds were  
19 monitored by the Optogait system (described in the methods section), and there was a close  
20 correlation between OptoGait measurements and the pre-determined (calculated) walking  
21 speeds. For the TFA group, correlation coefficients for the measured and calculated walking  
22 speeds of PWS minus 25 and 12.5 %, the PWS, and the PWS plus 12.5 and 25 % s, were:  
23 0.997 ( $p < 0.001$ ), 0.992 ( $p < 0.001$ ), 0.994 ( $p < 0.001$ ), 0.997 ( $p < 0.001$ ) and 0.998 ( $p <$   
24 0.001), respectively. For the CON group the correlation coefficients for the same speeds were:

1 0.984 ( $p < 0.001$ ), 0.989 ( $p < 0.001$ ), 0.985 ( $p < 0.001$ ), 0.993 ( $p < 0.001$ ) and 0.997 ( $p <$   
2 0.001), respectively.

### 3 *Oxygen uptake (Table 1)*

4 The oxygen uptake ( $\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) following walking with different speeds is shown in table  
5 1. There was no significant *interaction* between *group\*time*  $F(4,56) = 0.572$ ,  $p = 0.684$ ,  $\eta^2 =$   
6 0.039, but there was a significant *main effect* of *time*  $F(4,56) = 89.537$ ,  $p < 0.001$ ,  $\eta^2 = 0.865$   
7 upon oxygen uptake. There was no significant *main effect* of *group* on oxygen uptake  $F(1,14)$   
8  $= 1.027$ ,  $p = 0.328$ ,  $\eta^2 = 0.068$ , hence, the oxygen uptake for the TFA and CON was similar at  
9 all speeds. Mean group differences (confidence interval) for oxygen uptake was 1.403 (-1.566  
10 - 4.371).

11 Pairwise post-hoc comparisons (for time) with Bonferroni corrections showed that for the  
12 TFA, the oxygen uptake at the PWS was significantly higher compared to PWS minus 25% ( $p$   
13  $< 0.001$ ) and PWS minus 12.5% ( $p < 0.05$ ) and lower compared to PWS plus 25% ( $p <$   
14 0.001). For the CON, the oxygen uptake at the PWS was significantly higher compared to  
15 PWS minus 25% ( $p < 0.001$ ) and PWS minus 12.5% ( $p < 0.05$ ) and lower compared to PWS  
16 plus 12.5% ( $p < 0.05$ ) and PWS plus 25% ( $p < 0.001$ ).

17

### 18 *Percent $\dot{V}O_{2\text{max}}$ utilization (Table 1)*

19 There was no significant *interaction* between *group\*time*  $F(4,56) = 1.406$ ,  $p = 0.244$ ,  $\eta^2 =$   
20 0.091, but there was a significant *main effect* of *time*  $F(4,56) = 75.747$ ,  $p < 0.001$ ,  $\eta^2 = 0.844$   
21 and *group*  $F(1,14) = 14.259$ ,  $p < 0.01$ ,  $\eta^2 = 0.505$  upon %  $\dot{V}O_{2\text{max}}$ . Mean group difference  
22 (confidence interval) for percent  $\dot{V}O_{2\text{max}}$  utilization was 22.936 (9.909 -35.964).

1 Pairwise post-hoc comparisons (for time) with Bonferroni corrections showed that for the  
2 TFA, the %  $\dot{V}O_2$ max utilization at the PWS was significantly higher compared to PWS minus  
3 25% ( $p < 0.001$ ) and PWS minus 12.5% ( $p < 0.01$ ) and lower compared to PWS plus 25% ( $p$   
4  $< 0.001$ ). For the CON, the %  $\dot{V}O_2$ max utilization at the PWS was significantly higher  
5 compared to PWS minus 25% ( $p < 0.01$ ) and lower compared to PWS plus 25% ( $p < 0.001$ ).

6

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

8 There was no significant *interaction* between *group\*time*  $F(4,56) = 1.107$ ,  $p = 0.362$ ,  $\eta^2 =$   
9  $0.073$ , but there was a significant *main effect* of *time*  $F(4,56) = 3.180$ ,  $p < 0.05$ ,  $\eta^2 = 0.185$  and  
10 *group*  $F(1,14) = 29.873$ ,  $p < 0.001$ ,  $\eta^2 = 0.681$  upon the ECW.

11 Across the different walking speeds, the range of ECW values for the TFA and the CON were  
12  $0.213 - 0.226$  and  $0.146 - 0.174$ , respectively. Hence, since oxygen uptake and walking speed  
13 change more or less in parallel, the within-group ECW values show only small changes across  
14 the range of walking speeds. Pairwise post-hoc comparisons with Bonferroni corrections  
15 showed that the ECW of the CON was significantly lower compared to the TFA at the all  
16 speeds (all comparisons,  $p < 0.001$ ). For both the TFA and CON, the ECW at the PWS was  
17 similar to the ECW at the other walking speeds.

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

19 For carbohydrate, there was no significant *interaction* between *group\*time*  $F(4,56) = 0.576$ ,  $p$   
20  $= 0.681$ ,  $\eta^2 = 0.039$ , but there was a significant *main effect* of *time* upon carbohydrate  
21 oxidation rates  $F(4,56) = 41.225$ ,  $p < 0.001$ ,  $\eta^2 = 0.746$ . There was no significant *main effect*  
22 *of group* on carbohydrate oxidation rates  $F(1,14) = 0.477$ ,  $p = 0.328$ ,  $\eta^2 = 0.037$ .

1 At similar relative walking speeds, the mean difference in carbohydrate oxidation rates  
2 between the TFA and CON was small, and varied between 1 and 10 cal kg<sup>-1</sup> min<sup>-1</sup>. For both  
3 the TFA and CON, the carbohydrate oxidation rates at the PWS was significantly higher  
4 compared to PWS minus 25% (both, p < 0.05) and lower compared to oxidation rates at PWS  
5 plus 25% (p < 0.001).

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

9 At similar relative walking speeds, the fat oxidation rates of the TFA and CON were quite  
10 similar and mean difference in fat oxidation rates were in the order of 2 - 7 cal kg<sup>-1</sup>·min<sup>-1</sup>. Fat  
11 oxidation rates are shown in figure 2.

12

## 1 Discussion

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

17 Our results also show that carbohydrate and fat oxidation rates are similar for the TFA and  
18 CON when walking with similar relative speeds in the fed state. Thus, in the fed state there is  
19 little support for the hypothesis that the central nervous system naturally select a walking  
20 speed that require little or no net carbohydrate depletion<sup>12</sup>. Generally, the present study show  
21 that in the fed state, the preferred walking speed of the TFA is a *metabolic cross-over speed*<sup>23</sup>  
22 meaning that when the TFA walking speed exceeds the preferred walking speed, the  
23 carbohydrate oxidation rates increases more steeply and there is a concomitant reduction in  
24 the fat oxidation rates (Fig 2). A similar response is seen for the CON, and at the same  
25 relative speeds, the curves are virtually overlapping with the TFA curves. However, at the

1 preferred walking speed, the TFA and CON walk at an absolute speed of 73 and 91 m·min<sup>-1</sup>,  
2 thus the metabolic cross-over speed of the CON is at a higher absolute speed compared to the  
3 TFA. Furthermore, while the oxygen consumption at the preferred walking speed is similar  
4 (Table 1), the TFA utilized about 55 % of their  $\dot{V}O_2$ max, while the CON used about 31 % of  
5 their  $\dot{V}O_2$ max. Thus, the physical effort of walking at the preferred walking speed (PWS) is  
6 substantially greater for the TFA compared to the CON. Thus, when healthy persons walk at a  
7 leisurely pace (i.e. ~90 m·min<sup>-1</sup>), persons with a lower limb amputation struggle to keep the  
8 same pace, because in order to do so, they will need to increase their speed by about 25 %.  
9 The consequences of increasing the speed to 90 m·min<sup>-1</sup> (PWS plus 25%), is that the TFA  
10 utilized 71 % of their  $\dot{V}O_2$ max (Table 1) and close to 80 % of the total energy expenditure  
11 was provided by carbohydrate oxidation (Fig 2). In contrast, at the same absolute speed, the  
12 CON utilized only 31 % of their  $\dot{V}O_2$ max and about 55 % of their energy expenditure was  
13 provided by carbohydrate oxidation. At 90 m·min<sup>-1</sup>, the oxygen uptakes are similar for the  
14 TFA and CON and the energy cost of walking for both groups are unchanged compared to the  
15 preferred walking speed. This underscores that the energy cost of walking is not a good  
16 indicator of the physical effort of prosthetic ambulation.

17 As can be observed in figure 2, there is a great reliance on carbohydrate oxidation during fast  
18 walking and it is well established that endurance capacity and carbohydrate availability is  
19 highly interrelated<sup>24</sup>, hence the size of the carbohydrate stores in the body may be of special  
20 importance for persons with a lower limb amputation. Pertaining to this, persons with a  
21 transfemoral amputation evidently have less lower extremity muscle mass than healthy  
22 persons and many are untrained and have low aerobic capacity<sup>16, 25</sup>. The carbohydrate  
23 (glycogen) stores in skeletal muscles is limited by the quantity of muscle mass and the  
24 intrinsic glycogen storage capacity of muscle tissue (80-150 mmol·kg<sup>-1</sup> wet weight)<sup>26</sup>.  
25 However, skeletal muscle of untrained individuals may store only 50 % of the capacity of

1 trained skeletal muscle<sup>27-29</sup>. Thus, it is plausible that an untrained muscle mass and  
2 corresponding low intramuscular carbohydrate stores may considerably limit the endurance  
3 capacity and walking range of persons with lower limb amputation.

4 Furthermore, it is important to note that the rates of fat oxidation in the study of Willis et al.<sup>12</sup>,  
5 is higher than the fat oxidation rates in the present study. In the above study, fat accounted for  
6 about 65 % of the total energy expenditure during walking with the preferred walking speed,  
7 and in a similar study of persons with post-stroke hemiparesis, fat supplied 58 % of the total  
8 energy expenditure when walking with the preferred walking speed<sup>30</sup>. In the present study, fat  
9 utilization was about 45 % of the total energy expenditure for TFA and CON when walking  
10 with the PWS, hence fat combustion was substantially lower than in the studies of Willis et al.  
11 <sup>12</sup> and Ganley et al.<sup>30</sup>.

12 The reason for these discrepancies, may be related to the fact that the participants in the above  
13 studies were tested following an overnight fast, and it is expected that fat oxidation rates are  
14 quite high following prolonged fasting (> 6 hours)<sup>31</sup>. On the other hand, to investigate fuel  
15 oxidation during normal living, it is important to study the energy metabolism also in the non-  
16 fasted state, as in the present study. The following example illustrates the impact of prior  
17 feeding on fuel partitioning: In the Willis et al.<sup>12</sup> study, the total energy expenditure of  
18 healthy persons during treadmill walking with the treadmill preferred walking speed, was 63  
19 cal min<sup>-1</sup> kg<sup>-1</sup>, and carbohydrate and fat utilization accounted for 33 % and 67 % of the total  
20 energy expenditure, respectively. Oxygen uptake was 13.2 mL min<sup>-1</sup> kg<sup>-1</sup>, and heart rate was  
21 95 beats min<sup>-1</sup>. In a previous study in our lab<sup>32</sup>, using the same participants as in the present  
22 study, we observed that during treadmill walking at the preferred walking speed, CON mean  
23 oxygen uptake, heart rate and total energy expenditure was 13.4 mL min<sup>-1</sup> kg<sup>-1</sup>, 92 beats min<sup>-1</sup>  
24 and 68 cal min<sup>-1</sup> kg<sup>-1</sup>, i.e. similar to the Willis study<sup>12</sup>. However, carbohydrate and fat  
25 oxidation was 64 % and 36 % of the total energy expenditure, respectively and consequently,



1 the fuel partitioning in the fed state (present study) was reversed with regard to the fasted  
2 state<sup>12</sup>. This is, however, not unexpected as feeding suppresses fat oxidation during physical  
3 activity<sup>31</sup>. Thus, to enable persons with a lower limb amputation to adopt a faster walking  
4 speed and at the same time expend less energy, it is of importance that these persons perform  
5 aerobic endurance exercise. Regular endurance exercise will over time improve the maximal  
6 aerobic capacity ( $\dot{V}O_2\text{max}$ ) and switch substrate utilization towards fat oxidation.<sup>33</sup> This may  
7 result in a clinically relevant reduction in the relative oxygen uptake during prosthetic  
8 ambulation and a more sustainable substrate utilization. Consequently, this may possibly  
9 translate into functional improvements in walking speed, walking endurance and a reduction  
10 in the perception of the physical effort. The interrelationship between improved physical work  
11 capacity and gait performance is little investigated in lower limb amputees, but Wezenberg et  
12 al.<sup>9</sup> have developed a predictive quantitative model that show that even small increases in  
13 aerobic capacity can result in substantial improvements in walking ability of older adults with  
14 a lower limb amputation.

## 15 **Conclusion**

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

25

1 **Possible limitations**

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

8 *WORD COUNT: 4 000*

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3 **Declaration of Conflict of interests**

4 The authors declare that there is no conflict of interest.

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8 **Preparation of manuscript**

9 All authors contributed equally in the preparation of this manuscript

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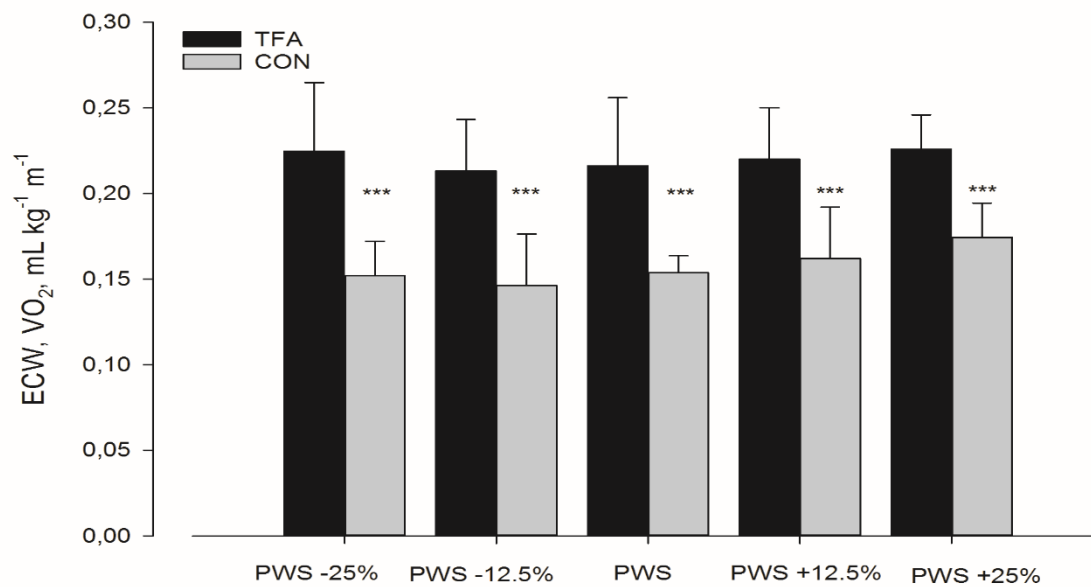
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- 1 **Figure 1.** Energy cost of walking (ECW) of the TFA and CON during walking with similar  
2 relative speeds.



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- 4 **Legend figure 1.** Values are means  $\pm$  SD. \*\*\*  $p < 0.001$ , TFA compared to CON.

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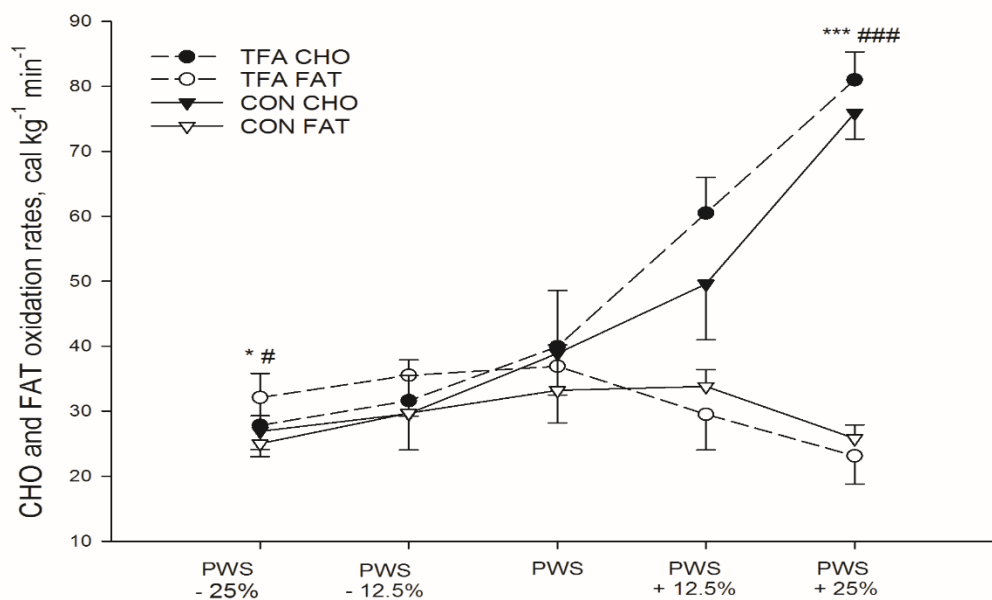
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2 **Figure 2.** Fuel oxidation rates of the TFA and CON during walking on the floor with different  
3 speeds.



4

#### 5 Legend Figure 2

6 Values are means  $\pm$  SD. \*\*\*  $p < 0.001$ , CHO oxidation rates at the PWS of the TFA  
7 compared to other speeds. ###  $p < 0.001$ , CHO oxidation rates at the PWS of the CON  
8 compared to other speeds.

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1 **Table 1.** Oxygen uptake and percent utilization of  $\dot{V}O_{2\max}$  at different walking speeds

Relative Speeds	Group	Oxygen uptake, $\text{mL min}^{-1} \text{kg}^{-1}$		Oxygen uptake, $\% \dot{V}O_{2\max}$	
		Means	95 % CI	Means	95 % CI
PWS minus 25%	CON	10.5 <sup>***</sup>	9.10 – 11.8	22.8 <sup>**</sup>	15.2 – 30.6
	TFA	12.2 <sup>***</sup>	10.9 – 13.5	42.3 <sup>***</sup>	34.7 – 49.9
PWS minus 12.5%	CON	11.7 <sup>*</sup>	9.5 – 14.1	25.5	17.8 – 33.2
	TFA	13.6 <sup>*</sup>	11.2 – 15.9	46.4 <sup>*</sup>	38.8 – 54.1
PWS	CON	14.1	11.9 – 16.2	30.9	21.3 – 40.5
	TFA	15.9	13.8 – 18.1	54.5	44.9 – 64.1
PWS plus 12.5%	CON	16.7 <sup>#</sup>	13.8 – 19.7	36.7	25.9 – 47.6
	TFA	17.8	14.8 – 20.7	61.0 <sup>#</sup>	50.1 – 71.8
PWS plus 25%	CON	20.0 <sup>###</sup>	17.4 – 22.6	44.2 <sup>###</sup>	32.1 – 56.3
	TFA	20.6 <sup>###</sup>	17.9 – 23.2	70.7 <sup>###</sup>	58.2 – 82.7

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3 **Legend Table 1.**

4 PWS = preferred walking speed, CI = confidence interval. \* $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p <$   
5  $0.001$ ; PWS compared to lower speeds. #  $p < 0.05$ , ###  $p < 0.001$ ; PWS compared to faster  
6 speeds.

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