Physiological Responses to Load Carriage During Level and Downhill Treadmill Walking

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Abstract

Introduction: Load carriage using backpacks is undertaken recreationally and as an occupational task. We assessed physiological changes during 2 hours of load carriage during level and downhill treadmill walking.

Methods: Ten male participants (age: 30±8 years, body mass: 79.4±8.3 kg, VO2 max: 55.1±5.6 ml kg⁻¹ min⁻¹) completed randomly 3 walking tests (6.5 km h⁻¹) for 2 hours: (1) level walking no load (LW), (2) level walking with a 25 kg backpack (LWLC) and (3) downhill walking (-8%) with a 25 kg backpack (DWLC).

Results: VO2 was higher during LWLC compared to LW at baseline (minute 5) (23.0±2.7 vs. 16.4±0.7 ml kg⁻¹ min⁻¹, P<0.001) and 120 minute (26.9±3.3 vs. 17.9±0.5 ml kg⁻¹ min⁻¹, P<0.001). The increase in VO2 during LWLC was greater over the 120 minutes (3.9±2.3 vs. 1.6±0.6 ml kg⁻¹ min⁻¹, P=0.018). VO2 during DWLC was lower than LWLC at baseline (17.1±1.6 vs. 23.0±2.7 ml kg⁻¹ min⁻¹, P<0.001) and minute 120 (21.4±3.0 vs. 26.9±3.3 ml kg⁻¹ min⁻¹, P<0.001), with no difference in VO2 increase over time (4.3±2.5 vs. 3.9±2.3 ml kg⁻¹ min⁻¹, P=0.411). Cardiovascular drift occurred between 5 and 120 minutes for LW (96±10 vs. 101±15 beats.min⁻¹, P=0.005), LWLC (116±13 vs. 141±23 beats.min⁻¹, P=0.001) and DWLC (103±9 to 126±21 beats.min⁻¹, P=0.001). RER decreased between 5 and 120 minutes during LWLC only (0.90±0.09 to 0.83±0.04, P=0.021). Stride frequency increased between 5 and 120 minutes during DWLC only (64±3 to 66±4 steps min⁻¹, P=0.043).

Conclusion: Differences in VO2 and cardiovascular drift between prolonged unloaded and loaded level treadmill walking and prolonged loaded level and downhill treadmill walking appear to relate to changes in substrate oxidation, muscle fatigue/damage and mechanical efficiency.

Key words: VO2 drift, cardiovascular drift, backpack, muscle fatigue, muscle injury

Introduction

Load carriage using backpacks is undertaken recreationally (1) and as an occupational task (2). Physiological effects of short duration load carriage in field and laboratory conditions have been well documented (2). During short duration load carriage, oxygen uptake (VO2) has been shown to increase with speed, load, and uphill gradient (3). In addition, increases in VO2 and heart rate during exercise have been shown to increase with backpack load mass (4). However, few studies have examined the change in VO2 during prolonged load carriage (i.e. ≥2 hours).

Epstein et al. (5) showed that when a 40 kg load was carried in a backpack at 4.5 km h⁻¹ on a +5% gradient for 120 min, VO2 increased between 20 and 120 minutes from 52.1 ± 0.6 to 56.2 ± 0.6 % of maximal oxygen uptake (VO2 max), respectively. This increase was not apparent when carrying a 25 kg load under the same conditions. Epstein et al. (5) suggested that VO2 drift occurred when the work rate was increased above 50% VO2 max. However, more recent data does not support this hypothesis. During a 12 km walk at 0% gradient, increases in VO2 have been shown with loads of 31.5 kg at 5.7 km h⁻¹ (36.6 ± 1.5 to 40.4 ± 2.0% VO2 max), 49.4 kg at 4.0 km h⁻¹ (26.6 ± 0.8 to 29.7 ± 0.9% VO2 max) and 49.4 kg at 5.7 km h⁻¹ (41.7 ± 1.1 to 50.1 ± 1.7% VO2 max), but not when walking unloaded at any speed (6). However, Sagiv et al. (7) found no increase in VO2 during 240 min of walking at 4.5 km h⁻¹ carrying 38 kg and 50 kg. It was suggested that, compared with previous studies, this may have been due to an improved backpack design which utilised hip and chest belts and shoulder straps allowing better mechanical efficiency (7). Further examination of Sagiv et al. (7) data shows that participants had relatively high maximum oxygen uptake values (VO2 max 65.2 ± 5.0 ml kg⁻¹ min⁻¹) and walked at a relatively low speed (4.5 km h⁻¹) carrying loads of 38 kg and 50 kg resulting in VO2 of only 14 ± 4 and 19 ± 5 ml kg⁻¹ min⁻¹, respectively.

It is unlikely that individuals would have been exercising above lactate threshold during the prolonged load carriage tasks discussed above. The lactate threshold has been shown to vary between 61% and 81% VO2 max in trained individuals (8), which is above the highest exercise work rate of 56.2 ± 0.6% VO2 max observed during 120 minutes of load carriage (5). Also, Patton et al. (6) showed no change in blood lactate following 145 minutes carrying a 49.4 kg backpack.
walking at 50.1% VO₂max, indicating that participants were not working above lactate threshold during the load carriage task and operating in the moderate intensity domain.

Patton et al. (6) and Warber et al. (9) have also shown increases in heart rate (HR) during bouts of prolonged load carriage which is indicative of cardiovascular drift (10). However, Sagiv et al. (7) showed no cardiovascular drift when participants carried 38 kg and 50 kg during 240 minutes of treadmill walking at 4.5 km·h⁻¹. The similarities in changes in VO₂ and heart rate are unsurprising due to the relationship between the respiratory and cardiovascular system and the changes that occur during exercise (11).

There is limited research investigating the effect of negative (downhill) gradients on load carriage. During short duration load carriage (< 20 minutes) VO₂ is reduced when walking on a negative gradient compared to walking on a level (0%) gradient (12). The gradient resulting in the lowest VO₂ was reported to be -8% (12). Further decreases in gradient cause VO₂ to increase (up to -12% has been investigated) (12). These findings are similar to unloaded downhill walking where VO₂ is lowest between -6 and -15% (13). During prolonged walking on negative gradients, a greater VO₂ drift over time has been observed compared to level walking (14, 15). When walking on a negative gradient the supporting muscles (e.g. the quadriceps) perform eccentric contractions. Davies and Barnes (14) suggest that the additional recruitment of muscle fibres during eccentric contractions to maintain stability and position when walking may lead to an increased VO₂. The effect of load carriage on mechanical efficiency, as determined by VO₂ and heart rate during prolonged walking on negative gradients, is unknown.

Potential reasons for an increase in VO₂ and heart rate during prolonged exercise include increased blood lactate concentration (16), increased body temperature (10) and changes in substrate utilisation (17). Patton et al. (6) suggested that a factor which may be of particular importance during prolonged load carriage is a change in mechanical efficiency when carrying load. However, these variables have not been investigated during prolonged load carriage and their relationship with a potential VO₂ and cardiovascular drift has not been confirmed.

This study had two main aims: (1) to compare the physiological changes during 2 hours of treadmill walking (6.5 km·h⁻¹) with no load and load carriage (25 kg backpack) and (2) to investigate the physiological differences of 2 hours of load carriage (25 kg backpack) on level (0%) and a negative gradient (-8%). It was hypothesised that: (1) load carriage on a level gradient would cause a higher VO₂ and heart rate and increase VO₂ and cardiovascular drift compared to walking with no load and (2) load carriage on a -8% gradient (downhill) would reduce VO₂ and heart rate but increase VO₂ and cardiovascular drift compared to load carriage on a level gradient.

**Methods**

**Participants**

Ten healthy male participants (age 30 ± 8 years, height 1.79 ± 0.05 m, body mass 79.4 ± 8.3, body fat 15.1 ± 2.8%, maximal oxygen uptake (VO₂max) 55.1 ± 5.6 ml·kg⁻¹·min⁻¹) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study. Participants were instructed to refrain from any vigorous physical activity in the day prior to treadmill walking and avoid consumption of caffeine, sports drinks or food 2 hours prior to the commencement of the test.

**Preliminary Measures**

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) was measured whilst wearing shorts and underwear. Skinfold measurements were taken at the Biceps, Triceps, Sub Scapular and Iliac Crest on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm, the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at four anatomical sites using previously described methods (18, 19).

Participants completed an incremental exercise test to exhaustion on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK) to assess VO₂max. All data collection procedures are described in detail in the experimental protocol. The VO₂max protocol consisted of running at a speed of 9 km·h⁻¹ on a gradient of 1%; gradient increased by 1%·min⁻¹ during the first 5 minutes. Speed then increased at 0.1 km·h⁻¹ every 5 seconds until volitional exhaustion. In at least the last 3 minutes of the test, expired gases were collected in 1 minute samples using Douglas bags (Plysu Protection Systems Limited, Milton Keynes, UK). The final bag was only used if the collection time was at least 30 seconds and contained > 65 l of expired gas. Heart rate was monitored throughout the test and recorded at 5 s intervals. A capillary blood sample was taken 4 minutes after the end of
the test and plasma lactate concentration measured. Participants were deemed to have reached $\dot{V}O_2\max$ if they obtained at least two of the following criteria; an increase in $\dot{V}O_2$ of $<2.1$ ml·kg$^{-1}$·min$^{-1}$ between the last 2 expired gas collections, plasma lactate $>5.5$ mmol·L$^{-1}$ or respiratory exchange ratio (RER) $>1.15$ (20). All participants attained $\dot{V}O_2\max$.

**Experimental Protocol**

The study was a three way cross over randomised design, where each subject performed the following conditions on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK): (1) two hours level walking at 6.5 km·h$^{-1}$ and 0% gradient carrying no load [Level Walking (LW)], (2) two hours level walking at 6.5 km·h$^{-1}$ and 0% gradient carrying a 25 kg backpack [Level Walking with Load Carriage (LWLC)], (3) two hours downhill walking at 6.5 km·h$^{-1}$ and -8% gradient carrying a 25 kg backpack [Downhill Walking with Load Carriage (DWLC)]. A time period of at least 7 days was left between conditions. Walking speed was kept constant between conditions and the absolute load used reflects realistic occupational requirements (e.g. military load carriage). Whilst participants walked on the treadmill, the measures described below were taken at 5, 15, 30, 45, 60, 75, 90, 105, 120 minutes of exercise. Measurements recorded at minute 5 were taken as a baseline and changes over time were calculated from this time point. $\dot{V}O_2$ and cardiovascular drift (heart rate) were calculated as the difference between the values measured at 5 minutes (baseline) and 120 minutes.

**Metabolic Measures**

Two minute collections of expired gases were made using Douglas bags. The Douglas bags were flushed with room air and fully evacuated prior to gas collection. Respiratory gas fractions [oxygen ($O_2$) and carbon dioxide ($CO_2$)] were analysed (Series 1400 gas analyser, Servomex plc., Crowborough, UK) and volume of expired air measured (Harvard dry gas meter, Harvard Apparatus Ltd., Edenbridge, UK). The gas analyser was calibrated using a two point calibration: $O_2$ and $CO_2$ were zeroed using 100% nitrogen gas (Linde Gas UK Ltd., West Bromwich, UK); $O_2$ was spanned to 20.95% using room air and $CO_2$ was spanned to 5.66% using a known gas mixture (5.66% $CO_2$) (Linde Gas UK Ltd., West Bromwich, UK). To calibrate the gas meter room air was pumped through in 35 L increments up to 175 L using a 7 L syringe (Model 4900, Hans Rudolph Inc., Kansas City, USA). Known volume was plotted against measured volume to obtain a correction factor (1.021 to 1.034). Expired gas volumes were corrected (measured volume x correction factor). Volume of oxygen uptake ($\dot{V}O_2$), using the Haldane transformation, and respiratory exchange ratio (RER) ($\dot{V}CO_2/\dot{V}O_2$) were calculated. Data are presented as standard temperature (0°C) and pressure (100.3 kPa) of dry gas (STPD).

**Heart Rate (HR)**

Heart rate was recorded every 5 seconds using downloadable heart rate monitors (Polar Vantage NV, Polar Electro Oy, Kempele, Finland). Average heart rates were calculated over one minute time intervals.

**Plasma Glucose and Lactate**

Capillary blood samples were taken from the finger and analysed for plasma lactate and glucose (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, USA). No lysing agent was used in the analyser, therefore plasma (rather than blood) lactate and glucose was measured.

**Stride Frequency**

The number of steps taken in 1 minute was measured. Stride frequency was determined by counting the number of steps taken in one minute, using counts from one foot and recorded each time it made contact with the treadmill (to the nearest half step).

**Ratings of Perceived Exertion (RPE)**

Participants were asked to rate their level of perceived exertion on a 15 point Borg Scale (21). The baseline reading of RPE was taken at minute 5 and at 15 minute intervals until completion of the test.

**Additional Measures**

Participants were weighed immediately prior to, and following, the treadmill walks to determine changes in body mass. Participants consumed water ad libitum during the experiment and intake was recorded and accounted for in the calculation of post exercise body mass. Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK) and controlled using the laboratory air conditioning (South East Cooling Ltd., Bognor Regis, UK). No differences in environmental temperature were determined between conditions [21.31 ± 0.78°C (LW), 21.28 ± 1.06°C (LWLC), 21.11 ± 0.50°C (DWLC)].

**Statistical Analysis**

SPSS for windows V15 (SPSS, Chicago, Illinois) was used for statistical analyses. Distribution of the data was assessed using Kolmogorov-Smirnov test for normality. Data were normally distributed and differences between groups and over time were assessed using 2 way repeated measures ANOVA. When differences were observed they were examined using pre-planned paired t-tests. Comparisons were made between (1) LW vs. LWLC and (2) LWLC vs. DWLC,
to ensure only one variable (i.e. load or gradient) was changed between compared conditions. The results are presented as mean ± standard deviation (SD). Statistical significance was set a priori at \( P < 0.05 \).

**Results**

**Level Walking (LW) vs. Level Walking with Load Carriage (LWLC)**

\( \dot{V}O_2 \) during LWLC was 41 ± 17% higher than LW at minute 5 (23.0 ± 2.7 vs. 16.4 ± 0.7 ml·kg\(^{-1}\)·min\(^{-1}\), \( P < 0.001 \)) and 50 ± 19% higher at minute 120 (26.9 ± 3.3 vs. 17.9 ± 0.5 ml·kg\(^{-1}\)·min\(^{-1}\), \( P < 0.001 \)). There was a greater absolute increase in \( \dot{V}O_2 \) over the 120 minutes during LWLC compared to LW (3.9 ± 2.3 vs. 1.6 ± 0.6 ml·kg\(^{-1}\)·min\(^{-1}\), \( P = 0.018 \)) (Figure 1A). However, expressed as a percentage change from the baseline value (5 minutes), the change in \( \dot{V}O_2 \) over the 120 minutes was similar for LWLC and LW (10 ± 4 vs. 17 ± 10%, \( P = 0.68 \)) (Figure 1B).

Similarly, HR during LWLC was 25 ± 7% higher than LW at minute 5 (116 ± 13 vs. 93 ± 8 beats·min\(^{-1}\), \( P < 0.001 \)) and 43 ± 16% higher at minute 120 (141 ± 23 vs. 99 ± 12 beats·min\(^{-1}\), \( P < 0.001 \)). There was a greater increase in HR over the 120 minutes during LWLC (116 ± 13 to 141 ± 23 beats·min\(^{-1}\)) compared during LW (96 ± 10 to 99 ± 12 beats·min\(^{-1}\)) (\( P = 0.001 \)) (Figure 2). This was accompanied by a higher plasma lactate concentration during LWLC at minute 5 (1.40 ± 0.32 vs. 0.90 ± 0.30 mmol·L\(^{-1}\), \( P < 0.001 \)) and minute 120 (0.84 ± 0.25 vs. 0.56 ± 0.20 mmol·L\(^{-1}\), \( P < 0.001 \)) (Table 1). RPE was higher during LWLC at minute 5 (10 ± 2 vs. 8 ± 2, \( P = 0.001 \)) and increased over the 120 minute duration for both LWLC (10 ± 2 to 14 ± 2, \( P = 0.003 \)) and LW (8 ± 2 to 9 ± 2, \( P = 0.003 \)) (Table 1).

Plasma glucose concentration was higher during LWLC compared to LW at minute 5 (4.21 ± 0.45 vs. 3.69 ± 0.59 mmol·L\(^{-1}\), \( P = 0.026 \)) and minute 120 (4.40 ± 0.30 vs. 4.00 ± 0.44 mmol·L\(^{-1}\), \( P = 0.023 \)) (Table 1). There was no difference in RER between LWLC and LW at minute 5 (0.90 ± 0.09 vs. 0.86 ± 0.06, \( P = 0.099 \)). However, RER decreased during LWLC from 0.90 ± 0.09 to 0.83 ± 0.04 (\( P = 0.021 \)) which was not apparent during LW (0.86 ± 0.06 to 0.84 ± 0.07, \( P = 0.234 \)) (Table 1).

A greater reduction in body mass was measured following LWLC compared to LW (1.45 ± 0.16 vs. 0.81 ± 0.19 kg, \( P < 0.001 \)). Participants did consume more water during LWLC (0.42 ± 0.35 vs. 0.16 ± 0.21 L, \( P = 0.006 \), however, this was less than the reduction in body mass (\( P < 0.001 \)) (Table 1).

Stride frequency was 2 ± 2 steps higher during LWLC at minute 5 (64 ± 3 vs. 62 ± 3 steps·min\(^{-1}\), \( P = 0.029 \)) and 3 ± 2 steps higher at minute 120 (65 ± 3 vs. 62 ± 3 steps·min\(^{-1}\), \( P = 0.001 \)). As participants maintained the same walking speed this indicates that stride length was reduced during LWLC. There was no change in stride frequency between minute 5 and 120 for both LWLC (64 ± 3 to 65 ± 3 steps·min\(^{-1}\), \( P = 0.708 \)) or LW (62 ± 3 to 62 ± 3 steps·min\(^{-1}\), \( P = 0.708 \)) (Table 1).

![Fig. 1. (A) Oxygen uptake (B) Percentage change in oxygen uptake from baseline value (minute 5) during 120 minutes of treadmill walking at 6.5 km·h\(^{-1}\) (n=10) with level walking carrying no load (LW, ■), level walking carrying 25 kg backpack (LWLC, •) and downhill walking carrying a 25 kg backpack (DWLC, *). Symbols indicate that \( \dot{V}O_2 \) at 120 min was increased above baseline for LW (#), LWLC (*) and DWLC (†) (\( P < 0.05 \)).](image1)

![Fig. 2. Heart rate (beats·min\(^{-1}\)) during 120 minutes of treadmill walking at 6.5 km·h\(^{-1}\) (n=10) with level walking carrying no load (LW, ■), level walking carrying 25 kg backpack (LWLC, •) and downhill walking carrying a 25 kg backpack (DWLC, *). Symbols indicate that HR at 120 min was increased above baseline for LW (#), LWLC (*) and DWLC (†) (\( P < 0.05 \)).](image2)
Table 1. Physiological responses to treadmill walking at 6.5 km·h⁻¹ (n=10) with level walking carrying no load (LW), level walking carrying 25 kg backpack (LWLC) and downhill walking carrying a 25 kg backpack (DWLC). Data presented as mean ± SD, at 5 minutes (baseline) and 120 minutes (final).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>LW</th>
<th>LWLC</th>
<th>DWLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO₂ (ml·kg⁻¹·min⁻¹)</strong></td>
<td>5 min</td>
<td>16.4 ± 0.7</td>
<td>23.0 ± 2.7 ***</td>
<td>17.1 ± 1.6 ###</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>17.9 ± 0.5 †††</td>
<td>26.9 ± 3.3 *** †† † †</td>
<td>21.4 ± 3.0 ### †† † †</td>
</tr>
<tr>
<td><strong>VO₂ (% VO₂ max)</strong></td>
<td>5 min</td>
<td>30.0 ± 3.5</td>
<td>42.1 ± 5.5 ***</td>
<td>31.4 ± 4.0###</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>32.9 ± 4.2 †† † †</td>
<td>49.3 ± 7.5 *** †† † †</td>
<td>39.3 ± 7.4 ### †† † †</td>
</tr>
<tr>
<td><strong>Heart Rate (beats·min⁻¹)</strong></td>
<td>5 min</td>
<td>93 ± 8</td>
<td>116 ± 13 ***</td>
<td>103 ± 9 #</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>99 ± 12 †</td>
<td>141 ± 23 *** ††</td>
<td>126 ± 21 ‡ ‡ † †</td>
</tr>
<tr>
<td><strong>Respiratory Exchange Ratio</strong></td>
<td>5 min</td>
<td>0.86 ± 0.06</td>
<td>0.90 ± 0.09</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>0.84 ± 0.07</td>
<td>0.83 ± 0.04 †</td>
<td>0.85 ± 0.04 #</td>
</tr>
<tr>
<td><strong>Stride Frequency (steps·min⁻¹)</strong></td>
<td>5 min</td>
<td>62 ± 3</td>
<td>64 ± 3 *</td>
<td>64 ± 3</td>
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<td></td>
<td>120 min</td>
<td>62 ± 3</td>
<td>65 ± 3 **</td>
<td>66 ± 4 †</td>
</tr>
<tr>
<td><strong>Plasma glucose (mmol·L⁻¹)</strong></td>
<td>5 min</td>
<td>3.69 ± 0.59</td>
<td>4.21 ± 0.45 *</td>
<td>4.19 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>4.00 ± 0.44</td>
<td>4.40 ± 0.30 *</td>
<td>4.36 ± 0.53</td>
</tr>
<tr>
<td><strong>Plasma lactate (mmol·L⁻¹)</strong></td>
<td>5 min</td>
<td>0.90 ± 0.30</td>
<td>1.40 ± 0.32 ***</td>
<td>1.04 ± 0.27 #</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>0.56 ± 0.20 †</td>
<td>0.84 ± 0.25 ** † † †</td>
<td>0.92 ± 0.32</td>
</tr>
<tr>
<td><strong>RPE</strong></td>
<td>5 min</td>
<td>8 ± 2</td>
<td>10 ± 2 **</td>
<td>9 ± 2</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>9 ± 2 † †</td>
<td>14 ± 2 ** † †</td>
<td>13 ± 3 # † †</td>
</tr>
<tr>
<td><strong>Change in Body Mass (kg)</strong></td>
<td>Pre – Post</td>
<td>0.81 ± 0.19</td>
<td>1.45 ± 0.16 ***</td>
<td>1.39 ± 0.15</td>
</tr>
<tr>
<td><strong>Fluid Intake (L)</strong></td>
<td>Total</td>
<td>0.16 ± 0.21</td>
<td>0.42 ± 0.35 **</td>
<td>0.32 ± 0.34</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01 and *** P<0.001 indicate a difference between LW and LWLC; † P<0.05, †† P<0.01 and ††† P<0.001 indicate a difference between LW and LWLC; ‡ P<0.001. HR increased over time (P<0.001). In the first 3 stages, there was no difference between conditions (P=0.411) (Figure 1A). However, the percentage change in VO₂ from the baseline during the 120 minutes was greater for DWLC compared to LWLC (25 ± 15 vs. 17 ± 10%, P=0.027) (Figure 1b). When divided into four time periods (5-30, 30-60, 60-90, 90-120), the percentage increase between conditions was similar in the first 3 stages, but during the final 30 minute stage (i.e. 90-120 minutes) the percentage increase in VO₂ was greater for DWLC than LWLC (7 ± 6 vs. 2 ± 3%, P=0.044).

HR was 11 ± 9% lower during DWLC at minute 5 (103 ± 9 vs. 116 ± 13 beats·min⁻¹, P=0.003) and 11 ± 9% lower during DWLC at minute 120 (126 ± 21 vs. 141 ± 23 beats·min⁻¹, P=0.004). HR increased over time for both DWLC (103 ± 9 to 126 ± 21, P=0.001) and LWLC (116 ± 13 to 141 ± 23, P=0.001) but there was no difference between conditions (P=0.719) (Figure 2). When expressed as a percentage change from the baseline value (5 min), there was no difference in the change in heart rate over the 120 minutes between DWLC and LWLC (22 ± 14 vs. 22 ± 15%, P=0.936). There was no difference in RPE between DWLC and LWLC at minute 5 (9 ± 2 vs. 10 ± 2, P=0.094). RPE increased over the 120 min walk for both DWLC (9 ± 2 to 13 ± 3, P=0.008) and LWLC (10 ± 2 to 14 ± 2, P=0.003). However, at minute 120, RPE was lower during DWLC (13 ± 3 vs. 14 ± 2, P=0.014) (Table 1).

Plasma lactate concentration was lower during DWLC compared to LWLC at minute 5 (1.04 ± 0.27 vs. 1.40 ± 0.32 mmol·L⁻¹, P=0.021) but there were no differences at minute 120 (0.92 ± 0.32 vs. 0.84 ± 0.25 mmol·L⁻¹, P=0.477). Of note, plasma lactate declined between minute 5 and minute 105 for DWLC (1.04 ± 0.27 to 0.65 ± 0.18 mmol·L⁻¹, P=0.003) and LWLC (1.40 ± 0.32 to 0.83 ± 0.21 mmol·L⁻¹, P<0.001). However, during the final 15 minutes of DWLC only, plasma lactate concentration increased from 0.66 ± 0.18 to 0.92 ± 0.32 mmol·L⁻¹ (P=0.008) (Table 1).

There was no difference in stride frequency between DWLC and LWLC at minute 5 (64 ± 3 vs. 64 ± 3 steps·min⁻¹, P=1.000). However, over the duration of DWLC there was an increase in stride frequency from 64 ± 3 steps·min⁻¹ at minute 5 to 66 ± 4 steps·min⁻¹.
at minute 120 (P=0.043) indicating a shortening of stride length, which was not apparent during LWLC (Table 1). This is despite the fact that during DWLC two participants reduced their stride length by 1 and 3 steps-min⁻¹, suggesting that there was individual adaptation to the task (Table 1).

There was no difference in RER between DWLC and LWLC at minute 5 (0.86 ± 0.05 vs. 0.90 ± 0.09, P=0.128). RER did not change over the duration of DWLC (0.86 ± 0.05 (min 5) to 0.85 ± 0.04 (min 120), P=0.490). During LWRC RER decreased over the 120 minutes from 0.90 ± 0.09 to 0.83 ± 0.04 (P=0.021).

There were no differences in plasma glucose concentration between DWLC and LWLC at 5 minutes (4.19 ± 0.54 vs. 4.21 ± 0.45 mmol-L⁻¹, P=0.914) or 120 minutes (4.36 ± 0.53 vs. 4.40 ± 0.30 mmol-L⁻¹, P=0.836) (Table 1).

There were no differences between DWLC and LWLC for change in body mass (pre-post, corrected for fluid intake) (1.39 ± 0.15 vs. 1.45 ± 0.16 kg, P=0.556) or fluid intake during the treadmill walking (0.32 ± 0.34 vs. 0.42 ± 0.35 L, P=0.171) (Table 1).

Discussion

In the present study, observations were made during 2 hours of treadmill walking at 6.5 km-h⁻¹. Carrying a 25 kg backpack increased VO₂ and heart rate and caused a greater VO₂ and cardiovascular drift over time compared to unloaded walking. Carrying a 25 kg backpack on a -8% (downhill) gradient decreased VO₂ compared to a level gradient. However, a novel finding was that despite the lower VO₂ during exercise, the increase in VO₂ over time was similar on level and -8% gradients. These data confirm the following hypotheses. Firstly, load carriage on a level gradient causes a higher VO₂ and heart rate and increases VO₂ and cardiovascular drift compared to walking with no load. Secondly, load carriage on a -8% gradient (downhill) reduces VO₂ and heart rate but increases VO₂ and cardiovascular drift compared to load carriage on a level gradient.

Level walking carrying a 25 kg backpack increased VO₂ compared to walking unloaded, which is in agreement with previous findings (6, 22). The initial work rate during LWLC in the current study was 42.1 %VO₂ max, which is similar to that of participants walking at 6.0 km-h⁻¹ carrying a backpack of 31% of body weight (23.6 ± 3.6 kg) which elicited an initial work rate of 40 %VO₂ max (22).

The addition of carrying load on a level gradient caused a greater absolute increase in VO₂ over time. Epstein et al. (5) and Patton et al. (6) have previously shown that VO₂ drift increases with the load carried. However, Patton et al. (6) showed that with lighter loads (5.2 kg; mass of clothing), VO₂ and cardiovascular drift were not apparent during 12 km of treadmill walking at 5.7 km-h⁻¹. During unloaded level walking, the present study showed an increase in VO₂ over time. The reason for this is unclear; compared with LW in the current study, the participants of Patton et al. (6) study were of a similar aerobic fitness (55.1 ± 5.6 vs. 58.5 ± 1.5ml-kg⁻¹-min⁻¹) and were working at a similar initial exercise work rate (30.0 ± 3.5 vs. 29.5 ± 0.9 %VO₂ max). The main differences between the present study and Patton et al. (6) were the faster treadmill speed in the current study [6.5 km-h⁻¹versus 5.7 km-h⁻¹ (6)] and the 10 min rest period after each 50 minutes of exercise in the Patton et al. (6) study. Despite the faster treadmill speed, the initial exercise intensity in the present study (17.1 ± 1.6 ml-kg⁻¹-min⁻¹) was similar to Patton et al. (6) (i.e. 17.2 ± 0.5 ml-kg⁻¹-min⁻¹). However, it is likely that the rest period caused a reduction in body temperature and partial recovery of muscle fatigue; variables that are likely to contribute cardiovascular (10) and VO₂ drift (23). Therefore, the rest period imposed in Patton et al. (6) may account for the attenuation in VO₂ and cardiovascular drift during treadmill walking without a backpack. In the present study, participants did not receive a rest period and that may have resulted in the cumulative effect of 2 hrs of treadmill walking on rises in body temperature (10) and muscle fatigue (23), likely accounting for the VO₂ and cardiovascular drift.

VO₂ while carrying 25 kg walking downhill was lower than carrying 25 kg during level walking at minute 5 (17.1 ± 1.6 vs. 23.0 ± 2.7 ml-kg⁻¹-min⁻¹) and minute 120 (21.4 ± 3.0 vs. 26.9 ± 3.3 ml-kg⁻¹-min⁻¹). Santee et al. (12) showed a similar decrease in VO₂ when participants carried a 18.1 kg backpack at 4.8 km-h⁻¹ on 0 and -12% gradients (10.4 ± 0.8 vs. 15.6 ± 1.1 ml-kg⁻¹-min⁻¹). Despite the lower VO₂, the absolute increase in VO₂ over time was similar between LWLC and DWLC. When expressed as a percentage increase from baseline, the increase in VO₂ was higher whilst carrying load downhill. This observation has not previously been shown.

Epstein et al. (5) suggested VO₂ drift would only occur if individuals were working above 50% VO₂ max. However, Patton et al (6) found that exercise intensities as low as 26.5% VO₂ max caused a drift in VO₂. The data of the present study supports these more recent findings and show that VO₂ drift occurred when participants walked at 31.3 ± 0.89, 45.9 ± 2.6 and 35.2 ± 2.5% VO₂ max for LW, LWLC and DWLC, respectively. This suggests that the reason for VO₂ drift during load carriage may be more subtle than simply a function of the exercise work rate (% VO₂max) at which individuals are exercising.

Carrying a load requires additional force and control from the muscles to maintain movement and posture and has been shown to increase muscle fibre recruitment of the shoulders (24), trunk (25) and lower
The increase in muscle fibre recruitment will increase the demand for oxygen and therefore cause $\text{VO}_2$ to rise. This may in part account for the higher $\text{VO}_2$ during LWLC compared with LW.

During prolonged exercise muscle fibres become fatigued and/or damaged, reducing the force they are able to produce (27). To compensate, additional motor units are recruited to maintain movement on the treadmill at the required speed and to support the load. This additional recruitment will increase oxygen demand and may drive an upward drift in $\text{VO}_2$. The higher stride frequency during LWLC requires participants to take an average of an additional 2 steps (i.e. 2 stretch shortening cycles) per minute. The combination of the greater force required from muscle fibres and higher stride frequency when carrying a load will increase muscle fatigue and therefore $\text{VO}_2$ drift which may account for some of differences between the LWLC and LW. Although a quantitative measurement of fatigue was not taken, the qualitative ratings of perceived exertion were greater for LWLC compared to LW and were observed to increase over time in both conditions.

During prolonged walking, $\text{VO}_2$ drift has been observed at -5%, -15% and -25% but not on 0% gradients (15). Davies and Barnes (14) suggested that the increase in $\text{VO}_2$ when walking on negative gradients may be due to the recruitment of additional muscle fibres in the supporting muscles (i.e. quadriceps) to maintain stride length and position on the treadmill during eccentric contractions. Type II muscle fibres are preferentially recruited during eccentric muscle actions (28), and muscle fibres become fatigued and damaged more rapidly during eccentric compared to concentric exercise (29). Therefore, we speculate that during DWLC a greater number of type II fibres were recruited initially, which become fatigued and/or damaged more rapidly due to the more severe eccentric muscle actions. The demand for oxygen will increase as additional motor units are recruited and may be further increased by the progressive switch from type II to type I fibres. This could add to the greater percentage increase in $\text{VO}_2$ observed during DWLC, especially during the final 30 minutes of the treadmill walking. Studies of the mechanisms responsible for $\text{VO}_2$ drift during 45 minute bouts of downhill running concluded that muscle damage could not account for the increase in $\text{VO}_2$ (30). However, these studies used serum creatine kinase as a measure of muscle damage which has been shown to be a poor indicator of force losses of the muscle (31). More recently, decreases in running economy following downhill running have been shown to be accompanied by decreases in the ability of the knee extensors to produce force (maximal voluntary contraction) (32). Also, Dick and Cavanagh (23) showed a 10% increase in $\text{VO}_2$ during a 40 minute downhill run at 44% $\text{VO}_2\text{max}$ with a corresponding 23% increase in IEMG, but no change in $\text{VO}_2$ or IEMG during 40 minutes of level running at 66% $\text{VO}_2\text{max}$. The authors concluded that the $\text{VO}_2$ drift observed during downhill running was due to muscle fibre damage and the recruitment of additional muscle fibres. Following 4 hours of treadmill load carriage (3% grade, 5.6 km·h$^{-1}$, 29.6 kg backpack), Warber et al (9) showed that squat jump performance (maximum number of squats with 45.5 kg at 25 repetitions-min$^{-1}$, 75 squat maximum) decreased by 53% ($P<0.001$). Potentially, fatigue of leg, hip and back muscles may have occurred during load carriage and contributed to this loss in strength. These findings suggest that muscle fatigue or damage may be linked to the changes in $\text{VO}_2$ drift during prolonged load carriage. Future work is suggested to examine muscle injury resulting from prolonged load carriage.

During treadmill running, a greater metabolic cost has been shown when stride frequency deviates from optimum (33). More recently, during a 60 minute steady speed run (at individual 10 km race pace), Hunter and Smith (34) observed a 3% increase in $\text{VO}_2$ and a corresponding decrease of 1-2% in stride frequency. The decrease in stride frequency was attributed to fatigue during the run. Stride frequency increased during DWLC but stayed constant during the LW and LWLC. The change in stride frequency would have contributed to the increase in $\text{VO}_2$ during DWLC and may explain some of the differences in $\text{VO}_2$ drift from LWLC. Stride frequency is a very crude measure of gait and more subtle changes in walking pattern may have occurred but were not measured in this study.

Participants’ diets were not controlled between conditions; therefore discussions of substrate oxidation must be interpreted with significant caution. However, participants arrived in the laboratory in a rested state and did not consume any food or fluid (other than water) at least 2 hours prior to starting the treadmill walking. There was no change in plasma glucose concentration over time in any condition. However, during LWLC there was a decrease in RER indicating a change from carbohydrate to fat as an energy source (35); this was not apparent during LW and DWLC. This may suggest that endogenous glycogen stores were reduced to a greater extent during LWLC compared to the other conditions. Reduced glycogen stores has been associated with a decrease in running economy (36). This may contribute to the increased $\text{VO}_2$ drift during LWLC.

Carbohydrate produces 5.02 kcal·L$^{-1}\text{O}_2$ where as fat only produces 4.85 kcal·L$^{-1}\text{O}_2$ (35). Assuming a negligible contribution from protein metabolism, at the start of LWLC (RER=0.90) 64% and 36% of energy was derived from carbohydrate and fat respectively, providing 4.96 kcal·L$^{-1}\text{O}_2$. At 120 minutes (RER=0.83),
43% and 57% of energy was derived from carbohydrate and fat respectively, providing 4.92 kcal·L⁻¹O₂. Therefore, if VO₂ remained constant throughout the 2 hours of treadmill walking, to supply the same amount of energy at minute 120 as minute 5 would only require an additional 0.01 L·O₂·min⁻¹. VO₂ increased by 0.20 L·min⁻¹ between minute 5 and 120, respectively. Therefore, the additional O₂ requirements for fat oxidation could only account for 5% of the increase in VO₂. However, RER is only an indication of whole body substrate oxidation, therefore glycogen depletion may have occurred locally in individual muscle fibres (37). This could further contribute to muscle fatigue and increases in VO₂ drift during LWLC.

Legg et al. (38) suggested that during load carriage localised muscular discomfort is more likely to have an ischemic or anaerobic origin rather than limitations associated with aerobic processes. A combination of higher exercise work rate and the strain imposed on individual muscle groups is likely to account for the higher plasma lactate during LWLC compared to LW. Following a cycling based endurance training programme, a reduction in VO₂ drift was strongly correlated with a decrease in blood lactate across a range work heavy exercise intensities (r=0.81; P<0.001) (16). Plasma lactate decreased during the 120 minutes of LWLC and there was a strong positive relationship (calculated using Pearson’s correlation coefficient) between VO₂ drift and plasma lactate concentration (r=0.80; P=0.005) which was not apparent during LW (r=0.25; P=0.487). Plasma lactate concentration was lower during DWLC compared to LWLC, potentially due to the lower exercise work rate (% VO₂ max). During DWLC there was no change in plasma lactate concentration over the duration of the exercise due to the increase between 105 and 120 minutes and there was no relationship between VO₂ drift and plasma lactate (r=0.06; P=0.868). Similarly, no relationship was found between blood lactate concentration and VO₂ drift during downhill running (30). It appears plasma lactate concentration is related to the VO₂ drift observed during LWLC, but not during LW and DWLC. It is unclear as to whether lactate is responsible for, or as a result of the VO₂ drift during LWLC.

In all test conditions, a progressive increase in heart rate was observed over the 120 minutes (Figure 2), which is an indication of cardiovascular drift (10). This has been shown in other studies of prolonged load carriage (6, 9). Cardiovascular drift occurs due to a decrease in stroke volume and an increase in heart rate, associated with a rise in core body temperature (10). Core temperature was not measured in this study. During a 12 km walk (6 km·h⁻¹) carrying 30 kg load in 21-24°C ambient temperature, core body temperature and heart rate have been shown to increase by 1.5 ± 0.7 °C and 61 ± 17 beats·min⁻¹, respectively (39). Also, during load carriage (14 kg, 4.4 km·h⁻¹, 5% gradient) in the heat (35°C), corresponding rises in core body temperature and heart rate have been observed (40). These data suggest that an increase in core body temperature may have been responsible for the cardiovascular drift observed during the exercise. However, following a cycling based endurance training programme, Casaburi et al. (16) found no relationship between a reduction in VO₂ drift and rise in core body temperature across a range of work heavy exercise intensities (r=0.15 P>0.05). Therefore it is unlikely that the differences in VO₂ drift observed in the present study were affected by changes in core body temperature.

The decrease in body mass indicates sweat loss during all conditions. Although the limitations of drawing conclusions from estimating hydration status by measuring changes in body mass are acknowledged (41). Compared to LW, estimated sweat loss was higher during LWLC and was not matched by fluid replacement. This suggests that participants became progressively dehydrated which would result in a hyperosmotic hypovolemic state, reducing stroke volume, increasing heart rate and therefore cardiovascular drift (10). Estimated fluid loss was also greater than fluid intake during DWLC, which would exacerbate cardiovascular drift as discussed previously.

**Conclusion**

In conclusion, carrying load on a level gradient increased VO₂ and HR and caused greater absolute VO₂ and cardiovascular drift compared to carrying no load. Potential mechanisms for the differences observed include: increased muscle fibre recruitment (due to the load and muscle fatigue/damage), changes in substrate utilisation, accumulation of plasma lactate and increased core body temperature. Compared to a level gradient, carrying load on a -8% (downhill) gradient decreased VO₂ and HR but increased the percentage VO₂ drift. This is likely to be due to increased muscle fibre recruitment, muscle fatigue and/or damage, a change in stride frequency and mechanical efficiency. The higher VO₂ and cardiovascular drift increased the physiological strain during load carriage, which would be exacerbated with task duration. Further studies are required to explore the potential mechanisms for changes in VO₂ and cardiovascular parameters and the effects these may have on individuals when carrying loads.

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