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Men and women adopt similar walking mechanics and muscle activation patterns during load carriage



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ABSTRACT

Although numerous studies have investigated the effects of load carriage on gait mechanics, most have been conducted on active military men. It remains unknown whether men and women adapt differently to carrying load. The purpose of this study was to compare the effects of load carriage on gait mechanics, muscle activation patterns, and metabolic cost between men and women walking at their preferred, unloaded walking speed. We measured whole body motion, ground reaction forces, muscle activity, and metabolic cost from 17 men and 12 women. Subjects completed four walking trials on an instrumented treadmill, each five minutes in duration, while carrying no load or an additional 10%, 20%, or 30% of body weight. Women were shorter (p < 0.01), had lower body mass (p = 0.01), and had lower fat-free mass (p=0.02) compared to men. No significant differences between men and women were observed for any measured gait parameter or muscle activation pattern. As load increased, so did net metabolic cost, the duration of stance phase, peak stance phase hip, knee, and ankle flexion angles, and all peak joint extension moments. The increase in the peak vertical ground reaction force was less than the carried load (e.g. ground force increased approximately 6% with each 10% increase in load). Integrated muscle activity of the soleus, medial gastrocnemius, lateral hamstrings, vastus medialis, vastus lateralis, and rectus femoris increased with load. We conclude that, despite differences in anthropometry, men and women adopt similar gait adaptations when carrying load, adjusted as a percentage of body weight.

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1. Introduction

Walking while carrying a load is a substantial component of military training and is associated with lower extremity injuries. In the year 2000, musculoskeletal injuries were termed a military epidemic (Jones et al., 2000), and they remain a leading health problem for service personnel (Cowan et al., 2003; Lee, 2011). Most previous studies aimed at understanding the effects of load carriage on gait mechanics and metabolic cost have been conducted on active military men (Attwells et al., 2006; Birrell and Haslam, 2009; Harman et al., 1992; Kinoshita, 1985; Knapik et al., 1997; Quesada et al., 2000), yet 15% of active military personnel are women (2011, 2012). Military personnel, regardless of sex, are often required to carry personal equipment and supplies (Knapik et al., 1997). When walking without load, gait kinematics and kinetics tend to be similar between men and women (Kerrigan et al., 1998); however, no study has investigated differences in

lower extremity gait kinematics and kinetics between men and women during load carriage.

Changes to gait mechanics during load carriage have been investigated by having subjects carry different magnitudes of load (Attwells et al., 2006; Birrell and Haslam, 2009; Knapik et al., 1997; Quesada et al., 2000) and different types of load (Bhambhani and Maikala, 2000; Kinoshita, 1985; Majumdar et al., 2010), often without controlling for walking speed. Some studies had all subjects carry the same fixed amount of load (Attwells et al., 2006; Birrell and Haslam, 2009; Knapik et al., 1997; Majumdar et al., 2010), while other studies had subjects carry loads as a percentage of body weight (Bhambhani and Maikala, 2000; Griffin et al., 2003; Holt et al., 2003; Kinoshita, 1985; Quesada et al., 2000). The distribution and type of load can affect spatiotemporal and kinematic gait patterns (Attwells et al., 2006; Majumdar et al., 2010) and metabolic cost (Birrell et al., 2007; Datta and Ramanathan, 1971; Knapik et al., 1997). Although allowing subjects to adjust their self-selected speed in response to the load is practical (Attwells et al., 2006; Majumdar et al., 2010), doing so makes it difficult to decouple the effects of load and walking speed. These variations in methodology may contribute to inconsistencies in the

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literature. For example, increased peak hip extension angle (Majumdar et al., 2010; Qu and Yeo, 2012) and stance phase knee flexion angle during load carriage have been observed by some studies (Attwells et al., 2006; Kinoshita, 1985; Quesada et al., 2000) but not others (Birrell and Haslam, 2009; Ghori and Luckwill, 1985; Holt et al., 2003; Pierrynowsi et al., 1981; Quesada et al., 2000). No study has reported how increasing load carriage alters gait mechanics in men and women while also controlling for walking speed and the type of load carried.

The effect of walking with load on muscle activity has also not been widely studied. Two separate studies examined muscle activation patterns in response to walking with load in female hikers (Simpson et al., 2011) and active military men (Harman et al., 1992). Interestingly, both studies reported that only quadriceps and gastrocnemius activity increased with load. Simulations of walking with 25% greater body weight (McGowan et al., 2010) also suggest that the quadriceps and gluteal muscles are the primary contributors to load acceptance and body weight support during the first half of stance phase. Walking with load also necessitates an increase in propulsive force during the second half of stance phase, which is provided primarily by the gastrocnemius and soleus (McGowan et al., 2010).

The purpose of this study was to investigate the biomechanical and physiological differences between men and women, when they were walking at a constant speed and carrying loads up to 30% of body weight. Subjects used weight vests, which mimic the mass distribution of body armor. We hypothesized men and women would adopt similar gait mechanics and muscle activation patterns when carrying load as a percentage of body weight. In support of previous studies, we expected that, when carrying load, men and women would both experience increased net metabolic cost (Pandolf et al., 1977; Pierrynowsi et al., 1981; Quesada et al., 2000), stance time (Birrell and Haslam, 2009; Harman et al., 1992; Wiese-Bjornstal and Dufek, 1991), and peak stance phase knee flexion angles (Attwells et al., 2006; Kinoshita, 1985; Quesada et al., 2000). To meet the increased demand for propulsive forces and body weight support with load we hypothesized that integrated lower extremity muscle activity of the plantarflexors (i.e. soleus and gastrocnemius) and three muscles of the quadriceps (i. e. vastus lateralis, vastus medialis, rectus femoris) would increase with load during stance phase.

2. Methods

2.1. Participants

Seventeen men $(31\pm7~{\rm years})$ and 12 women $(36\pm8~{\rm years})$ provided written informed consent to participate in this study according to a protocol approved by the Stanford University Institutional Review Board. All subjects were free of current or past injury. Each subject's body mass, percent body fat, and fat-free mass were measured using whole body dual-energy X-ray absorptiometry (iDXA; GE Healthcare, Waukesha, WI, USA). On average, the women were 10 cm shorter (men $1.79\pm0.07~{\rm m}$; women $1.69\pm0.08~{\rm m}$, p<0.01), 12 kg lighter (men $75\pm7~{\rm kg}$; women $63\pm7~{\rm kg}$, p=0.01), had a higher percent body fat (men $15\pm4\%$; women $20\pm6\%$, p=0.02), and lower fat-free mass (men $59\pm6~{\rm kg}$; women $45\pm5~{\rm kg}$, p<0.01) than the men participating in this study.

2.2. Experimental protocol

Prior to the experimental testing, subjects were asked to walk for 3–5 min on a treadmill and choose a preferred walking speed. All subsequent walking trials were performed on a split belt instrumented treadmill (Bertec Corporation; Columbus, OH, USA) at each subject's preferred level treadmill walking speed (men $1.28\pm0.07~\text{m/s}$; women $1.30\pm0.10~\text{m/s}$). Subjects completed four walking trials, each lasting 5 min. The trials were completed in random order while carrying no load (body weight, BW), or an additional 10%, 20%, or 30% of BW. Each 10% increase in load added $7.5\pm2.3~\text{kg}$ for the men and $6.3\pm1.6~\text{kg}$ for the women. Subjects carried loads using an adjustable weight vest (HyperWare, Austin, TX, USA). We chose this method of load carriage because it left the pelvis exposed to place

motion capture markers. Unlike heavy backpacks (Hasselquist et al., 2004) the weight vest resulted in a minimal change to the anterior–posterior center-of-mass location and lower metabolic cost compared to backpacks (Datta and Ramanathan, 1971; Patton et al., 1991).

2.3. Metabolic cost

Prior to the walking trials, standing metabolic cost was estimated by measuring oxygen consumption, $\dot{V}O_2$ (milliliters of O_2 s⁻¹), and carbon dioxide output, $\dot{V}CO_2$ (milliliters of CO_2 s⁻¹), for a minimum of 5 min until oxygen levels reached a plateau for at least 2 min (Quark b², Cosmed, Italy). Subjects were asked to refrain from caffeine and physical activity the morning of testing and to get a full night rest prior to testing. Steady state $\dot{V}O_2$ and $\dot{V}CO_2$ were analyzed during the final minute (minutes 4–5) of each walking trial. Gross metabolic cost during quiet standing and each walking trial was estimated from the steady-state $\dot{V}O_2$ and $\dot{V}CO_2$ (Brockway, 1987). To verify steady-state was achieved, we ensured that oxygen consumption during the final minute of each trial was within \pm 5% of the oxygen consumption during the previous minute. Standing involves the metabolic cost of body weight support, which is also required for walking (Weyand et al., 2009). Therefore, standing metabolic cost (1.37 \pm 0.33 W/kg) was subtracted from gross metabolic cost during walking to obtain the net normalized metabolic cost of walking.

2.4. Motion capture

Whole body motion (measured at 100 Hz) and treadmill forces (measured at 2000 Hz) were analyzed for five consecutive left limb gait cycles, which were collected during the final minute of each trial. Motion was measured using 40 retro-reflective markers with an eight-camera optical motion-capture system (Vicon, Oxford Metrics Group, Oxford, UK). Markers were attached bilaterally to anatomical landmarks on the upper limbs (medial and lateral elbow, wrist), trunk (acromium processes, sternoclavicular joints, and C7), pelvis (anterior superior iliac spines and posterior superior iliac spines), medial and lateral femoral condyles, the medial and lateral malleoli, and the foot (calcaneous, 5th metatarsal). An additional 15 markers were used to aid in segment tracking, making a total of at least three markers per segment. We used a scaled, 29 degree-of-freedom, 12 segment model to represent the torso, arms, pelvis, and lower extremity for each subject (Hamner et al., 2010). The pelvis was the base segment with six degrees-of-freedom, the hip was represented as a spherical joint with three degrees-of-freedom, the knee was represented as a one degree-of-freedom joint in which non-sagittal rotations and tibiofemoral and patellofemoral translations were computed as a function of the sagittal knee angle (Walker et al., 1988), and the ankle (talocrural) and subtalar joints were represented as pin joints aligned with the anatomical axes (Delp et al., 1990). Each segment was defined by a mutually orthogonal local coordinate system and defined according to the International Society of Biomechanics standards. An upright static calibration trial and functional hip joint center trial (Piazza et al., 2004) were used to define body segment coordinate systems, tracking marker locations, joint centers, and segment lengths for each subject.

A global optimization inverse kinematics routine was used to compute pelvis position, pelvis orientation, and lower extremity joint angles at each time frame in the trials; this method minimizes the effect of measurement error and soft tissue artifact (Lu and O'Connor, 1999). Body segment kinematics, anthropometric properties (de Leva, 1996), and treadmill forces were used to perform the investe dynamics analyses and compute lower extremity joint moments. To do this, we used SIMM Dynamics Pipeline (Motion Analysis Corp, Santa Rosa, CA, USA; (Delp and Loan, 2000)). All joint moments were divided by body mass, and step length and step width were normalized to height.

2.5. Muscle activity

Surface electromyography (EMG) electrodes were placed on the left soleus, medial gastrocnemius, tibialis anterior, medial hamstrings, lateral hamstrings, vastus medialis, vastus lateralis, and rectus femoris muscles according to Basmajian and De Luca (1985). Prior to electrode placement, skin was cleaned with alcohol and shaved. EMG signals were recorded at 2000 Hz with preamplified single differential, rectangular Ag electrodes with 10 mm inter-electrode distance (DE-2.1, DelSys, Inc, Boston, MA, USA). Signals were band-pass filtered (30-500 Hz, 4th order, Butterworth), full wave rectified, and passed through two additional filters: a 4th order 15 Hz critically damped filter and the Teager-Kaiser Energy operation (Li et al., 2007) (which included re-rectifying the data). EMG data were divided into, and averaged across, the same five gait cycles as the motion trials. Data passed through the critically damped filter were normalized to the maximum low-pass filtered signal of the respective muscle activity for each subject during walking with no load, and subsequently integrated across stance phase, swing phase, and the entire gait cycle. We used a critically damped filter to estimate the magnitude of muscle activity because it has a steeper roll-off, compared to a Butterworth filter (Robertson and Dowling, 2003). Data passed through the Teager-Kaiser Energy operation were used to determine the onset, offset, and duration of muscle activity according to Li et al., (2007). This method increases the signal-tonoise ratio, and improves the detection of muscle activity timing (Li et al., 2007). A threshold for muscle activity was manually chosen during a 100–200 ms time window when the muscle was inactive. The muscle was considered active during any time when the signal was greater than two standard deviations from the mean; the mask created by the threshold was manually checked for consistency and accuracy.

2.6. Statistics

The effects of sex and load on several groups of dependent measures were determined using repeated measures ANOVA, with the main effect of sex and load as fixed effects. Measures included the duration of stance phase, step length, cadence, step width, peak hip, knee, and ankle angles and moments, muscle activation patterns (magnitude and timing of the soleus, medial gastrocnemius, tibialis anterior, medial hamstrings, lateral hamstrings, vastus medialis, vastus lateralis, and rectus femoris muscles), and metabolic cost. Significance for all analyses was established at p < 0.05.

3. Results

No significant sex differences or sex-by-load interactions were detected for any spatio-temporal or kinematic measurements; we therefore report the mean \pm SD for combined male and female data (Table 1). Peak hip flexion, stance phase knee flexion, and ankle dorsiflexion angles increased with load (p < 0.05, Fig. 1). The duration of stance phase increased from 61 \pm 2% of the gait cycle during unloaded walking to 63 \pm 2% when carrying 30% of BW (p < 0.01). During unloaded walking, step length, step width, and cadence were 40 \pm 3% of height, 14 \pm 1% of height, and 112 \pm 4 steps/min respectively; these did not change with load.

No significant sex differences or sex-by-load interactions were detected for joint kinetics or peak vertical ground reaction forces divided by body mass; we therefore report combined male and female data (Table 1). With the exception of the peak hip flexion moment, the magnitude of all peak joint moments increased with

load (p < 0.05, Fig. 1). Peak vertical ground reaction forces during loading and pushoff increased by an average of ~6% and ~5%, respectively, with each 10% increase in load (p < 0.01, Fig. 2).

No significant sex differences or sex-by-load interactions were detected for any muscle activation parameter when activation was normalized to the peak activation during unloaded walking; we therefore report combined male and female data. Muscle activity integrated across the entire gait cycle increased with load for the soleus, gastrocnemius, lateral hamstrings, vastus medialis, vastus lateralis, and rectus femoris (p < 0.05). With the exception of the rectus femoris, muscle activity of this same set of muscles increased with load during stance phase (p < 0.05, Fig. 3). Only tibialis anterior activity showed no significant effect of load. The only change in muscle activation timing occurred in the rectus femoris, which stayed active for 5% longer during the first half of swing phase when subjects carried 30% of BW (p = 0.04).

Mean net normalized metabolic cost during unloaded walking was 3.21 ± 0.58 W/kg for the men and 2.80 ± 0.60 W/kg for the women. Metabolic cost averaged across men and women increased ~8% with each 10% increase in load (p < 0.01, Fig. 4). When normalized to body mass, the net metabolic cost of walking was greater for men at all loading conditions, compared to women (p < 0.01). When net metabolic cost was normalized to fat-free mass, there was no significant difference between men and women (p = 0.30).

4. Discussion

The purpose of this study was to investigate the biomechanical and physiological adaptations of men and women while carrying loads up to 30% of body weight and walking at their preferred unloaded walking speed. We hypothesized that men and women

Table 1
Mean (SD) kinematic, and kinetic measurements as subjects walked while carrying no load (body weight, BW) and an additional 10%, 20%, and 30% of BW. No sex differences or sex-by-load interactions were detected. Therefore all data except are reported are pooled across all male and female subjects. Reported p-values represent a main effect of load and a main effect of sex.

	Mean (SD) pooled across all male and female subjects				p-value	
	BW	10%	20%	30%	load	sex difference
Spatiotemporal measures						
Stance phase (%)	60 (2)	60 (2)	61 (1)	62 (2)	< 0.01	0.40
Stride length (% height)	40 (3)	40 (3)	40 (3)	40 (3)	0.61	0.16
Cadence (steps/min)	112 (4)	112 (6)	112 (5)	112 (6)	0.63	0.16
Step width (% height)	14 (1)	14 (1)	14 (1)	14 (1)	0.23	0.82
Peak joint kinematics (deg)						
Hip						
Flexion	29 (5)	30 (6)	30 (5)	32 (5)	< 0.01	0.28
Extension	-16 (7)	-16 (7)	- 17 (6)	- 16 (6)	0.09	0.54
Knee Flexion			(-)			
Stance	22 (5)	23 (6)	24 (6)	26 (6)	< 0.01	0.36
Swing	70 (5)	70 (5)	71 (4)	71 (6)	0.09	0.46
Ankle	. ,	. ,	` '	. ,		
Dorsiflexion	13 (4)	13 (4)	13 (4)	14 (4)	0.01	0.57
Plantarflexion	- 12 (5)	- 13 (6)	- 12 (5)	- 12 (5)	0.23	0.73
Peak Joint Moments (Nm/kg)						
Hip						
Flexion	0.60 (0.20)	0.62 (0.21)	0.66 (0.23)	0.67 (0.21)	0.24	0.30
Extension	-0.99(0.24)	-1.08 (0.22)	- 1.14 (0.21)	-1.23 (0.30)	< 0.01	0.47
Knee	` ,	, ,	, ,	, ,		
Flexion	0.47 (0.17)	0.52 (0.18)	0.55 (0.21)	0.57 (0.26)	< 0.01	0.57
Extension	-0.96(0.22)	-1.00 (0.21)	- 1.17 (0.27)	-1.28(0.30)	< 0.01	0.20
Ankle	,	,	, ,,	(,		
Plantarflexion	- 1.63 (0.19)	- 1.81 (0.25)	-1.91 (0.20)	-2.06 (0.24)	< 0.01	0.31
Peak vertical ground reaction fo	rce (N/kg)					
Loading	1.16 (0.10)	1.21 (0.14)	1.29 (0.17)	1.33 (0.19)	< 0.01	0.28
Pushoff	1.07 (0.07)	1.13 (0.14)	1.18 (0.26)	1.25 (1.16)	< 0.01	0.73

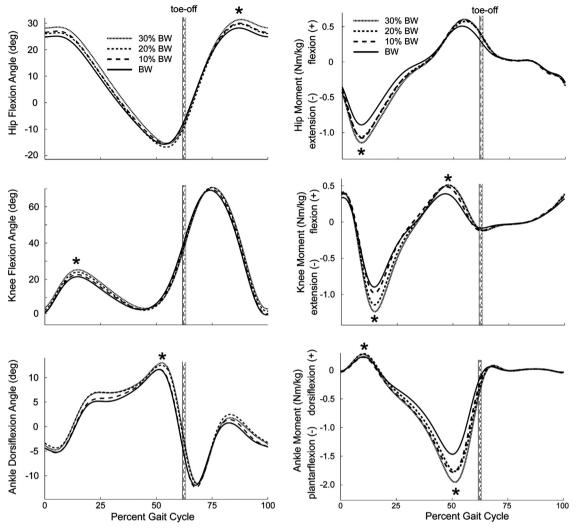


Fig. 1. Hip, knee, and ankle kinematics and moments while carrying no load (BW), and an additional 10%, 20%, and 30% BW. Asterisks (*) represent a significant main effect of load on the peak joint angle or moment (p < 0.05). Vertical lines indicate the end of stance phase, which increased significantly with load.

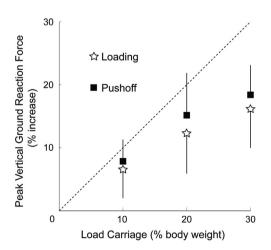


Fig. 2. The percent increase in peak vertical ground reaction force during loading (first half of stance) and pushoff (second half of stance) was less than the added load. The dotted line represents an equal increase in peak vertical ground reaction force with added load, and the vertical lines represent the standard deviation of the mean peak vertical ground reaction force.

would adopt similar gait mechanics and muscle activation patterns when carrying load as a percentage of body weight. Consistent with this hypothesis, we did not detect any significant differences in gait mechanics or muscle activation patterns between men and women. In support of our second hypothesis, we found that for both men and women, carrying load resulted in an increase in net metabolic cost, stance time, and stance phase knee flexion angles. In support of our final hypothesis, integrated lower extremity muscle activity of the soleus, gastrocnemius, and vasti increased with load during stance phase.

We did not detect significant differences in stance time, step length, step width, cadence, or peak hip, knee, and ankle angles and moments between men and women walking with load. In agreement with previous studies, we measured longer stance times (Birrell and Haslam, 2009; Harman et al., 1992; Wiese-Bjornstal and Dufek, 1991) and increased peak stance phase knee flexion angles as load increased (Attwells et al., 2006; Bastien et al., 2005; Birrell and Haslam, 2009; Kinoshita, 1985; Quesada et al., 2000). Longer stance times in conjunction with increased stance phase knee flexion can help to lower the first peak of the vertical ground reaction force. We found that the percent increase in peak ground reaction force was less than the percent increase in added load (Fig. 2). It has been suggested that increasing knee flexion during the first half of stance phase acts as a protective measure to help absorb impact forces (Attwells et al., 2006), and reduces injury risk during prolonged load carriage (Attwells et al., 2006; Harman et al., 1992; Kinoshita, 1985). However, walking with knee joint flexion requires greater

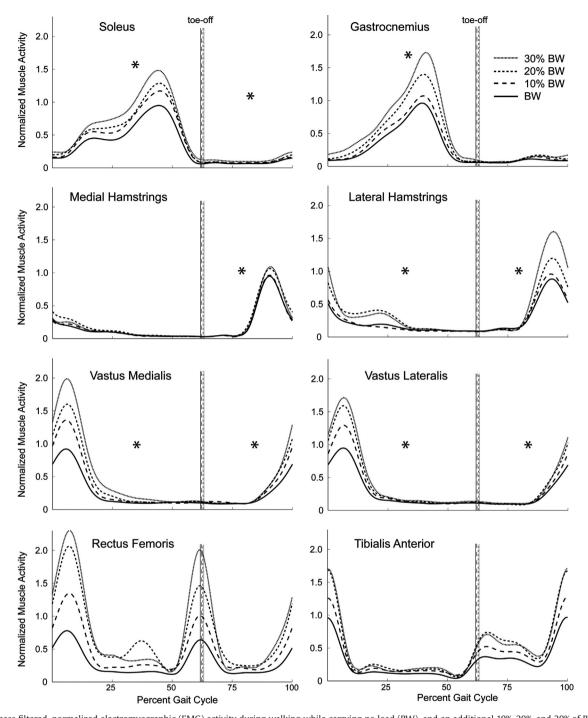


Fig. 3. Low-pass filtered, normalized electromyographic (EMG) activity during walking while carrying no load (BW), and an additional 10%, 20%, and 30% of BW. Data were normalized to the maximum low-pass filtered signal of the respective muscle activity for each subject during walking with no load. Muscle activity across the gait cycle increased with load for the soleus, gastrocnemius, lateral hamstrings, vastus medialis, vastus lateralis, and rectus femoris. Asterisks (*) represent a significant increase in integrated EMG activity during the stance and/or swing phase of the gait cycle (p < 0.05). No significant sex differences were detected in the magnitude muscle activity during stance or swing phase, respectively (soleus, p = 0.99, p = 0.11; gastrocnemius, p = 0.85, p = 0.14; medial hamstrings, p = 0.19, p = 0.51; lateral hamstrings, p = 0.49, p = 0.63; vastus medialis, p = 0.62, p = 0.74; vastus lateralis, p = 0.99, p = 0.33; rectus femoris, p = 0.42, p = 0.69; tibialis anterior, p = 0.46, p = 0.47). Therefore, data were averaged and curves are presented as pooled across all men and women in this study.

muscle activity (Steele et al., 2010), which increases metabolic cost (Waters and Mulroy, 1999) and joint contact force (Steele et al., 2012). It is the increased activity of lower limb muscles during stance phase that likely accounts for the increased metabolic cost of load carriage.

The magnitude of the second peak of the ground reaction force is also affected by the stance phase gait mechanics. Simulations show that the soleus and gastrocnemius muscles are largely

responsible for generating the second peak of the ground reaction force and accelerating the center of mass during late stance (Liu et al., 2006; McGowan et al., 2010; Neptune et al., 2001). Our results, which show significant increases in soleus and gastrocnemius activity (Fig. 3), support these simulation results. Simulations of loaded walking (McGowan et al., 2010) also suggest that as load increases, the vasti and rectus femoris are the primary muscles responsible for producing greater forces needed for body

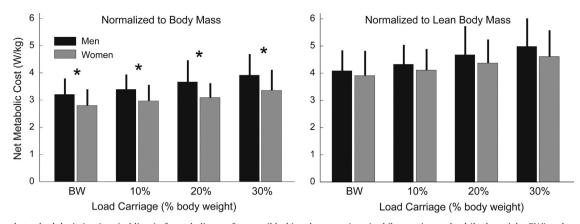


Fig. 4. Mean and standard deviation (vertical lines) of metabolic cost for men (black) and women (gray) while carrying no load (body weight, BW) and an additional 10%, 20%, and 30% of body weight. Metabolic cost increased significantly with load for both sexes. When normalized to body mass, women had a lower net metabolic cost of walking than men at all load carriage conditions (p < 0.05). We did not detect any sex differences when net metabolic cost was divided by lean body mass (p = 0.30). * indicates a sex difference at each load carriage condition.

weight support during the first half of stance phase, which concurs with our findings of increased stance phase activity of the vasti and rectus femoris.

When normalized to body mass, women had a significantly lower net metabolic cost during all load carriage conditions, but when normalized to fat-free mass the net metabolic cost of walking was not significantly different between men and women. This was not entirely unexpected as prior studies have found body composition to be correlated with the net metabolic cost of walking (Browning et al., 2006; Lyons et al., 2005). Our results are in agreement to those of Hall et al., (2004) who found that the energetic cost of walking was similar between men and women when normalized to fat-free mass.

Carrying load as a percentage of body mass and normalizing gait parameters and muscle activation patterns enabled us to investigate differences between men and women during load carriage, while reducing the effects of body size. However, many gait patterns are related to body mass and height. For example, regardless of sex, the first peak in the ground reaction force during unloaded walking was highly correlated with body mass (r=0.868, p<0.01). The results of our study suggest that there are no differences in normalized gait mechanics between men and women. It is important to note that comparing absolute measures between men and women carrying a fixed amount of load would be likely to reveal differences between men and women, perhaps due to differences in anthropometry.

Subjects in this study carried load using an adjustable weight vest, which distributes mass on the front and back, similar to a double pack or body armor. Wearing a backpack instead of a double pack reduces the ground force produced during pushoff (Birrell and Haslam, 2009) and shifts the center of mass posteriorly. Compared to a backpack, the double pack also causes fewer deviations from normal walking patterns (Kinoshita, 1985) and positions the mass closer to the trunk, which lowers metabolic cost (Datta and Ramanathan, 1971; Patton et al., 1991). It is likely that carrying a backpack with the mass shifted more posteriorly, would result in gait adaptations different from those measured in our study.

It is also important to note that our measurements were taken under steady state conditions. As fatigue occurs, the energy cost of load carriage increases (Epstein et al., 1988) and individuals may adopt different muscle activation strategies and gait kinematics (Qu and Yeo, 2012).

This study provides measurements of gait kinematics, gait kinetics, muscle activity patterns and metabolic cost for men and women walking with up to 30% body weight. These data serve as a

foundation for future studies aimed at identifying the mechanical determinants of metabolic cost of load carriage and are available online (http://www.simtk.org/LoadCarriage).

Conflict of interest

We, the authors have no conflicts of interest with regard to this manuscript and the data presented therein.

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