SKELETAL MUSCLE METABOLISM AFTER STROKE: A COMPARATIVE STUDY USING TREADMILL AND OVERGROUND WALKING TESTS

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Objective: The primary aim of this study was to investigate muscle metabolism in stroke survivors through measurements of the respiratory exchange ratio and rates of fat and carbohydrate oxidation in relation to total energy expenditure at preferred walking speed during treadmill and overground walking. The secondary objective was to investigate whether the energy source used during walking influences the daily physical activity pattern and fatigue of post-stroke individuals.

Methods: The sample comprised 28 stroke participants and 10 non-disabled, healthy controls. Measurements of oxygen consumption and carbon dioxide production were recorded. Participants wore a uniaxial accelerometer (activPAL™) over 4 days as an estimate of daily physical activity. Measurements of Human Activity Profile and Neurological Fatigue Index for stroke were documented.

Results: Carbohydrate oxidation accounted for the majority of fuel oxidation at preferred walking speed in the stroke group (55.86% vs 47.29% during treadmill walking and 66.13% vs 50.15% during overground walking). Stroke patients who had higher levels of carbohydrate oxidation reached a lower score in the Human Activity Profile survey, had fewer steps screened by activPAL data (4,422 vs 6,692 steps/day) and higher fatigue index.

Conclusion: Carbohydrate oxidation accounted for the majority of fuel oxidation at the preferred walking speed in post-stroke individuals. The increased carbohydrate utilization recorded at preferred walking speed may have influenced the physical activity profile.

Key words: stroke; energy expenditure; physical activity; fatigue.

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Stroke survivors often exhibit residual neurological deficits that impair function and mobility, predisposing them to risk of developing patterns of inactivity that may contribute to deconditioning, fatigue and functional loss (1). Independent walking ability and functional independence in activities of daily living are essential factors for improved quality of life and integration in the community (2). A higher energy cost of walking (ECW) in chronic post-stroke individuals has been linked to reduced walking performance compared with able-bodied individuals (3–5), and self-selected gait speed may be half of normal values (6). In healthy individuals, the preferred walking speed (PWS) is remarkably constant, between 1.1 and 1.3 m/s, and is related to minimal metabolic cost (7). Increased walking energy cost is inversely related to the walking speed naturally adopted by individuals and that the PWS represents the nadir of energy economy of a U-shaped curve that reflects the relationship between gait speed and energy cost of transport (8–10). Walking speed has also been related to specific fuel utilization by skeletal muscle (11, 12). In non-disabled individuals, the influence of exercise intensity on substrate partitioning is fairly well understood (13). During walking activity, the contribution of fat and carbohydrate (CHO) oxidation to total energy expenditure (TEE) is modulated by the intensity of physical activity. In a larger study with healthy elderly subjects, 60–80 years of age, CHO and fat utilization were 48% and 52%, respectively, when the participants were walking at their PWS (14). In general, the fractional contribution of CHO to the fuel supply is small at low intensity and rises with the energy turnover rate (15). The fuel selected by working skeletal muscle profoundly impacts endurance capacity (12, 16, 17).

To date, to the best of our knowledge, the total energy expenditure and substrate oxidation during walking at an early post-stroke stage has not been studied cross-sectionally. Therefore, the primary aim of the present study was to investigate muscle metabolism through measurements of the respiratory exchange ratio (RER) and rates of fat and CHO oxidation in relation to TEE during walking on treadmill (TM) and overground (OG) in the sub-acute post-stroke phase, compared with healthy, matched controls. The secondary aim...
was to investigate whether the energy source during OG influences fatigue and the daily physical activity pattern of post-stroke individuals.

**METHODS**

**Study participants**

A total of 28 stroke subjects took part in this study. Subjects were consecutively selected at Vestre Viken Hospital Trust, Bærum Hospital, Bærum, Norway, on acute admission to the stroke unit between August 2013 and January 2014 and patients were included according to the following criteria: (i) primary diagnosis of first ischaemic or haemorrhagic stroke, clinically and radiologically confirmed; (ii) a score on the modified Rankin Scale between 0 and 4 points; and (iii) Mini-Mental State Examination (MMSE) score of 23 or more. Exclusion criteria were: (i) evidence of coexisting known malignant or other rapidly progressively medical diseases; (ii) significant aphasia; (iii) severe cardiac arrhythmia; (iv) congestive heart failure; (v) unstable angina; (vi) resting blood pressure above 180/110 mmHg; (vii) nursing home residents; and (viii) individuals unable to provide informed consent. The control group included 10 volunteers, recruited from the same local community, matched with the stroke group according to age, sex and body mass index. Controls were free of any neurological disease and had no existing medical problems limiting their physical activity. Individuals who met the criteria were given a detailed explanation of the study and were asked to sign the consent form approved by the Regional Committee for Medical Research Ethics (REK) number 2013/383.

**Study design**

The study was cross-sectional, observational and descriptive. Participants who spent 8 days at the acute hospital were recruited and underwent 2 evaluations. The first session, assessed during hospitalization, entailed personal factors, such as age, sex and risk factors. In addition, stroke characteristics and the National Institutes of Health Stroke Scale (NIHSS) were taken. At the second session, 4 weeks after discharge from the hospital, outcome measurements of Human Activity Profile, Fatigue Stroke Scale and physical tests were performed. Participants reported to the laboratory for physical testing, and performed 2 tests. First, a walking test on the treadmill, followed by a walking test on the floor, both recorded after advice given for performance of PWS, assessing measures of walking speed (m/min), oxygen uptake (O₂), respiratory exchange (RER) and percentage utilization of CHO and fat. All participants were asked to abstain from drinking tea or coffee prior to the walking tests. In addition, their body composition was analysed with a bioelectrical impedance device, and they were fitted with an activity monitor for measurement of ambulatory physical activity. All participants with stroke were actively undergoing inpatient rehabilitation post-stroke. Demographic and clinical data are shown in Table I.

**Physiological measurements**

During both treadmill and floor testing, steady state oxygen uptake respiratory exchange ratio (RER) and heart rate (HR) were monitored with a portable breath-by-breath gas analyser (Metamax 3B, Cortex Biophysik, Germany). Data were continuously monitored by real-time telemetry to verify steady state conditions. Each walking interval lasted 7 min to enable the participants to reach steady state conditions (18) and data reported on physiological measurements are mean values calculated from the last 2 min of each walking interval. The oxygen analyser was calibrated for barometric pressure and gas, with 2 reference gas mixtures (16% O₂, 4% CO₂ and 26% O₂, 0% CO₂), according to the manufacturer’s instructions. In addition, a volume calibration was performed using a standardized 3 L syringe. Rating of perceived exertion (RPE) scores (6–20) were recorded prior to each walking interval and immediately following termination of the walk interval. Energy cost of walking (ECW; ml/kg/m) was calculated by dividing the participants O₂ consumption (ml/kg/min) during walking by the respective walking speed (m/min). A lower ECW value signifies a better walking economy.

**Procedure: treadmill walking experiment**

Walking trials at the participant’s treadmill preferred walking speed (PWS) were performed on a calibrated Woodway ELG 70 treadmill (Woodway, Weil am Rhein, Germany) with no inclination (0°). The PWS was determined according to an established protocol (19). The subjects were blinded for the actual walking speeds and no verbal feedback of walking speeds was given to the subjects during the determination of the PWS.
Once the preferred speed was chosen, the test lasted for 7 min. Participants were authorized to rest one hand on the handrail on the treadmill to stabilize balance. Following the treadmill test, the participants rested as long as necessary to return to a basal heart rate, oxygen consumption and no muscle fatigue prior to the start of their overground test.

Procedure: floor walking experiment

The participants were asked to walk at their floor walking PWS in a continuous loop for 7 min around a 20-m length rectangular room marked by cones at each end, whilst whole-body substrate oxidation was measured using a portable gas analyser. On the floor, the walking speed was monitored by an optical gait analysis system (OptoGait, Microgate, Bolzano-Bozen, Italy). Verbal instructions were given if the participants needed to adjust their walking speed during floor trials. In addition to the OptoGait registrations, the walking speed was manually checked by registering the time taken to walk 10.0 m along the indoor track.

Body composition analysis

Basic anthropometric data (weight, body mass index, waist-hip ratio) were assessed by electrical bioimpedance (InBody720, InBody Company, Seoul, Korea). Human Activity Profile (HAP). The HAP includes a survey of 94 activity items that require increasing energy expenditure from low to high activity levels. For each item the participant indicates if they are still doing the activity, have stopped doing the activity, or if they never did the activity. Their highest level activity that they are still doing on the scale is noted and represents their maximum activity scores (MAS). Their adjusted activity score (AAS) is calculated by subtracting the total number of activities the individual has stopped doing from those they are still doing. Higher scores represent greater activity. The HAP has been shown to be valid and reliable in the stroke population (20).

Neurological Fatigue Index for stroke (NFI-Stroke). The NFI-Stroke represents a combination of physical and cognitive components, in a brief (12-item), easy-to-use tool for measurement of a clearly defined concept of fatigue. The NFI-Stroke has specific validation for stroke and can be used on patients of any age, or sex, amongst other factors (21).

ActivPAL™ Ambulatory physical activity was assessed using the validated activPAL™ (PAL Technologies Ltd, Glasgow, UK) (22). This device has been used to investigate free-living physical activity in stroke survivors (23). The activPAL is a small (53 × 35 × 7 mm), lightweight (15 g) device that uses a uniaxial accelerometer to sense limb position and activity. The activPAL records the start and stop time of each individual bout (or event) of activity and classifies an individual’s free-living activity into periods spent sitting or lying (defined as sedentary behaviour), standing and walking and the number of steps (defined as physically active behaviour). The activPAL was attached after the laboratory walking tests to the anterior aspect of the participant’s non-paretic mid-thigh by a non-allergenic hydrogel adhesive (PALstickies™, USA), using a 10 × 12 cm transparent film dressing (Opsite Flexigrid – Smith & Nephew/ Medical Ltd, UK). Participants were asked to record 24 h/day including showering and sleeping time, totalling 4 registered days (3 week days and 1 weekend day).

Comparison of treadmill vs overground walking at preferred walking speed

The study included 28 stroke patients and 10 age-matched control subjects. Walking speed was significantly lower on the TM compared with OG (p = 0.001; p = 0.001) for stroke and control subjects, respectively. It is noteworthy that the mean speed of the stroke group was lower than that of the control group in the TM (p = 0.002) and OG (p = 0.004) test. Although the study group had a greater walking speed OG compared with TM, lung ventilation volume, perceived exertion and heart rate did not differ between groups for any of the surfaces. Stroke subjects showed significantly larger increases in energy cost of walking (ECW) when walking on the TM compared with OG (p < 0.001). In contrast, there were no differences in ECW between TM and OG in healthy controls (p = 0.06) or when compared with post-stroke individuals OG (p = 0.42). Compared with the control group, VO2peak was lower in the stroke group in both environments (TM = 11.31 (SD 2) vs 14.58 (SD 2); OG = 11.30 (SD 2) vs 14.45 (SD 2) ml/kg/min). RERs were similar between the 2 groups on the TM (p = 0.50) and OG (p = 0.17), but showed significant difference between the environments in the stroke group (p = 0.005). Despite the fact that rates of CHO and fat oxidation were similar for TM walking (p = 0.05), significant differences were found between group means OG, where stroke participants had higher rates of CHO oxidation (p = 0.016) and lower rates of fat (p = 0.016). Metabolic response data are shown in Table II.

Physical activity profile

Mean NFI-Stroke scale scores were significantly higher in the stroke group (p = 0.006), whereas the means of HAP (72.5 (SD 13.9) vs 38.2 (SD 27.1)) and total number of steps/day (mean 6,692 (SD 2,249) vs mean 4,422 (SD 3,104)) were significantly higher in the control group (p = 0.001 and p = 0.041, respectively). Association analysis revealed negative correlation between HAP × CHO (r = 0.42; p = 0.029), HAP × NFI-Stroke (r = 0.41; p = 0.034) and positive correlation between HAP × speed and number of steps, respectively (r = 0.57; p = 0.003; r = 0.51; p = 0.009). VO2 peak values revealed significant correlation with HAP (r = 0.44; p = 0.020); speed (r = 0.53; p = 0.004); number of steps (r = 0.45; p = 0.016) and negative correlation with RPE post (r = 0.56; p = 0.003). The results concerning EWC showed only negative correlation with speed (r = 0.64; p = 0.001). No association of these variables was found in the control group, except VO2 peak × speed (r = 0.74; p = 0.014) and RPE post × NFI-Stroke (r = 0.63; p = 0.049).
In the current study, CHO oxidation accounted for the majority of fuel oxidation at PWS in post-stroke individuals, in contrast to the controls. It is thought that the utilization of CHO increases with intensity of effort (15). Willis et al. (12) have suggested that the central nervous system may be guided by the perception of effort in selecting a PWS that minimizes dependence on CHO oxidation. In this respect, the percentage utilization of fat and CHO were similar for stroke participants and controls on the TM and, broadly speaking, the percentage utilization of fat and CHO was approximately 50/50. In contrast, CHO was the dominant fuel source and provided approximately 70% of the energy needed at the PWS during OG walking. In comparison, for the control group, only 50% of the energy was provided from CHO, which was significantly less than for the stroke participants. Thus, the stroke participants must rely heavily on CHO oxidation during walking conditions, where able-bodied individuals, otherwise, mostly oxidize fatty acids in accordance with previous reports (14). CHO utilization upwards of 70% will probably indicate more of an anaerobic walking bout for many of the stroke participants, and potentially limits the capacity to expand walking. To the best of our knowledge, there is only 1 study that describes muscle metabolism or fuel utilization in persons with chronic stroke and matched controls during OG walking at PWS (11). In the study by Ganley et al. (11), fuel oxidation was similar between groups, and fat was the dominant fuel source in PWS, providing approximately 60% of the energy, in comparison with approximately 30% in our study. However, in the study by Ganley et al. (11), the PWS by the persons with stroke during OG walking was much slower (0.83 m/s) than in our study (1.05 m/s) and consequently fat oxidation would be higher (15). However, when the stroke participants in Ganley’s study walked at a speed of 1.00 m/s, the rate of CHO oxidation increased to 68%, similar to the findings in the present study. Consequently, it seems that fat metabolism may be compromised in persons with stroke.

It is known that the contribution of fat, as the primary source in aerobic metabolism, is influenced by muscle mass, muscle fibre type, capillary density, and alterations in the muscle’s oxidative capabilities (17). The lower capacity in oxygen extraction in stroke survivors could, in part, be attributed to the structural changes in skeletal muscle that may start as early as 4 h after cerebral infarction, and this may develop muscle weakness and atrophy within a few days even in the unaffected limb (24). An interesting study by De Deyne et al. (25) observed a shift in muscle fibre-types in a population of persons with chronic stroke. This study observed a significant increase in the proportion of fast myosin heavy chain and a concomitant reliance on anaerobic metabolism (i.e. CHO oxidation), and this shift in muscle fibre-type properties was inversely associated with the severity of gait deficiency. Little is known about the adaptive responses in skeletal muscle tissue in the sub-acute phase after a stroke and it is not possible to conclude whether such alterations could occur in individuals with a mild stroke. However, this may help to explain the increased CHO utilization in the stroke participants in the present study.

### DISCUSSION

#### Table II. Metabolic response to walking on treadmill and overground

<table>
<thead>
<tr>
<th></th>
<th>Treadmill test</th>
<th>Overground test</th>
<th>p-value within-group Stroke</th>
<th>p-value within-group Control</th>
<th>p-value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>Stroke 95.7 (2) 105.3 (14)</td>
<td>Control 104.3 (22) 107.5 (13)</td>
<td>0.031</td>
<td>0.643</td>
<td>0.435</td>
</tr>
<tr>
<td>BV, l/min</td>
<td>Stroke 27.7 (6) 32.2 (11)</td>
<td>Control 29.6 (7) 35.5 (12)</td>
<td>0.145</td>
<td>0.007</td>
<td>0.406</td>
</tr>
<tr>
<td>VE, l/min</td>
<td>Stroke 11.2 (2) 10.8 (3)</td>
<td>Control 11.3 (2.5) 14.2 (3)</td>
<td>0.376</td>
<td>0.852</td>
<td>0.767</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.009</td>
<td>Control 0.012</td>
<td>0.005</td>
<td>0.699</td>
<td>0.226</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.30 (0.1) 0.20 (0.0)</td>
<td>Control 0.19 (0.0) 0.16 (0.0)</td>
<td>0.005</td>
<td>0.699</td>
<td>0.226</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.6 (0) 0.84 (0.0)</td>
<td>Control 0.9 (0.0) 0.85 (0.0)</td>
<td>0.005</td>
<td>0.699</td>
<td>0.226</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 56.2 (23) 47.7 (29)</td>
<td>Control 66.1 (23) 50.1 (26)</td>
<td>0.133</td>
<td>0.714</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.195</td>
<td>Control 0.016</td>
<td>0.133</td>
<td>0.717</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 43.7 (23) 52.2 (29)</td>
<td>Control 33.8 (23) 49.8 (26)</td>
<td>0.133</td>
<td>0.717</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.196</td>
<td>Control 0.016</td>
<td>0.133</td>
<td>0.717</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.7 (0.3) 1.2 (0.2)</td>
<td>Control 1.0 (0.3) 1.5 (0.2)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.486</td>
</tr>
<tr>
<td>CHO, %</td>
<td>Stroke 0.002</td>
<td>Control 0.004</td>
<td>0.133</td>
<td>0.717</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

VE: lung ventilation volume; RPE: rating of perceived exertion; VO2: oxygen uptake; ECW: energy cost of walking; RER: respiratory exchange ratio; CHO and FAT: percentage carbohydrate and fat utilization; ANOVA: analysis of variance. Significant values are shown in bold.
The stroke group had a faster PWS OG compared with on the TM, and this pattern was also followed by the healthy participants. The observed variance in gait speed may be partially explained taking into account that TM walking may provide an added challenge to balance as a result of altered visual, vestibular and propioceptive input, fear of falling and the constant belt-speed movements (26, 27). Despite being a more challenging activity in a less natural environment, TM training has been a recognized intervention for gait rehabilitation in the stroke population. TM walk training promotes a more symmetrical gait pattern (28), and the diversity of study protocols can present results are sampled during a single evaluation session and that the diversity of study protocols can make it difficult to compare results across studies.

The findings of this study showed a significant increase in speed in OG walking in the stroke subjects compared with on the TM. Similar findings with slower speeds on the TM compared with OG have been reported in some observational studies in individuals with sub-acute and chronic stroke, with or without weight support (31) in individuals using (26, 32) or not using handrail support (26, 27). Contrary to our findings, Bayat et al. (27) showed no speed change under either condition in the healthy age-matched control group.

In the present study, the oxygen uptake of the stroke participants was similar during TM and OG walking, but the walking economy, as evaluated with ECW, was considerably lower during OG walking. In fact, based on the ECW, and compared with TM, the energy cost of walking decreased by 63% during OG walking. This pattern was repeated for the control group, but with a higher magnitude of 80%. The walking speed of stroke participants was, however, considerably higher during OG walking. Since the calculation of ECW is considerably influenced by walking speed, it may not be correct to judge the physical effort of walking based on calculations of ECW. This view is consistent with the report by Detrembleur et al., which suggests that the energy cost is inversely related to the walking speed spontaneously adopted by the participants (3). This finding is also in agreement with Reisman et al. (33), who reported that with very slow self-selected walking speeds the economy improves as speed increases above the subjects’ initial self-selected speeds. On the other hand, Brouwer et al. (34) observed that with chronic stroke subjects (even walking at the same speed on TM and OG) the OG walking was less energy-demanding than unsupported TM walking (34). This observation reinforces the statement that the increase in energy cost is probably multifactorial. Other factors, such as fear and anxiety related to falls, compensatory mechanisms, and impaired balance could influence the energy requirement of walking after stroke (26). Consequently, it is necessary to use other indicators than the ECW for judging the physical effort of walking, especially if one is to make meaningful comparisons with able-bodied people. One way of doing this, may be by studying the percentage utilization of fat and CHO during ordinary walking.

**Physical activity**

The secondary objective of this study was to explore the possible association between muscle metabolism responses during the OG test and the level of daily physical activity. The study reports both self-reported and performance-based data monitored by a real-time accelerometer. The findings show that ambulatory stroke survivors are less physically active free-living than the healthy controls due to most of the participants having mild physical impairments. There are other reasons for reduced activity beyond severity of stroke deficiencies, and this study suggested that substrate oxidation during walking may also be related to reduced physical activity level in sub-acute stroke survivors. In our findings, people with stroke reported higher scores on the fatigue scale than matched controls, and these participants also showed higher levels of CHO oxidation during OG. However, we cannot say to what extent muscle metabolism influences the fatigue symptoms. Fatigue may contribute to a reduced ambulatory activity over time, especially if we take into account the fact that this debilitating symptom can increase during the first year post-stroke (35). The relationship of the multifarious components and the true impact of fatigue on stroke recovery remains uncertain (36). Studies of fatigue in the acute phase have suggested that poor functional outcome was more consistently associated with increased levels of fatigue (37, 38). This is in contrast to findings of other studies carried out in chronic phases reporting no significant relationship between fatigue and physical function (36). Presence of fatigue, one year after the stroke, was a significant predictor of mobility, which was most strongly associated with psychological and cognitive factors and not with physical factors (39). These data suggest that other factors, such as physiological measurements, should be properly identified and considered at early stages of rehabilitation. These findings may indicate that post-stroke fatigue can partly be explained by fuel oxidation, supporting metabolic functions involved in the aetiological mechanisms. Further research is needed to explore the supposed multifactorial mechanisms in post-stroke fatigue.
Study limitations

The sample size in this study was relatively small. The stroke participants had considerable disease burden (diabetes, hypertension, dyslipidaemia), as has been reported by other studies (40). However, there were no significant differences between participants in comorbidities, so this was not controlled for in the analyses. Despite the small sample size and the heterogeneous population, we were able to detect statistically significant results, suggesting that the results obtained reflect more generic principles regarding energy expenditure during physical activity.

Conclusion

This study demonstrated that CHO oxidation accounted for approximately 70% of the fuel oxidation at PWS of the stroke group. It seems that our stroke participants are not able to regulate their walking speed to a level of energy expenditure, which they could sustain for a long time. The increased CHO utilization may have influenced the physical activity profile of the stroke group, which showed a lower score in the HAP, fewer steps and higher score on the NFI scale. The findings of the current study could be viewed as indicative of the importance of preventing further deterioration of aerobic activity and helping to restore optimal performance in basic activities of daily living. Further research is needed to examine the role of the total energy expenditure and muscle substrate oxidation in the recovery phase of sub-acute stroke, in order to evaluate exercise as a therapeutic modality.

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The authors declare no conflicts of interest.

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