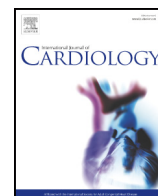




Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Longer-term impact of hemiparetic stroke on skeletal muscle metabolism—A pilot study

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ARTICLE INFO

Article history:

Received 28 August 2016

Received in revised form 19 December 2016

Accepted 20 December 2016

Available online xxxx

Keywords:

Stroke

Energy metabolism

Calorimetry

Microdialysis

Mitochondrial function

Muscle tissue perfusion

ABSTRACT

Background: Hemiparetic stroke leads to structural and metabolic alterations of skeletal muscle tissue, thereby contributing to functional impairment associated with stroke. *In situ* metabolic processes at tissue level in skeletal muscle have not been investigated. We hypothesize that muscular metabolic capacity is limited after hemiparetic stroke, and that changes affect rather the paretic than non-paretic limb.

Methods: Nine male hemiparetic stroke survivors (age, 62 ± 8 years; BMI, 28 ± 4 kg/m²; median stroke latency, 23 months ranging from 7 to 34 months poststroke) underwent dynamic *in situ* measurements of carbohydrate and lipid metabolism at fasting condition and during oral glucose tolerance testing, using bilateral microdialysis. Results were compared to 8 healthy male subjects of similar age and BMI.

Results: Tissue perfusion, fasting and postprandial profiles of interstitial metabolites glucose, pyruvate, lactate and glycerol did not differ between paretic and non-paretic muscle. Patients displayed higher fasting and postprandial dialysate glycerol levels compared to controls ($P < 0.001$) with elevated plasma FFA (fasting FFA; 0.63 ± 0.23 vs. 0.29 ± 0.17 mmol/L; $P = 0.004$). Glycolytic activity was higher in patients vs. controls, with increased lactate production upon glucose load ($P < 0.001$).

Conclusions: An elevated lipolytic and glycolytic activity on tissue level suggests an impaired substrate metabolism with blunted oxidative metabolism in bilateral skeletal muscle in patients after hemiparetic stroke. Muscular metabolic properties did not differ between paretic and non-paretic leg. Further work is needed to investigate the clinical impact of this impaired muscular metabolic capacity in post-stroke patients.

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Introduction

Stroke is the single greatest cause of long-term adult disability in modern society. Although its mortality has been declining in Western Europe and the USA over the last few decades, [1–3] prevalence of stroke survivors is additionally very likely to increase due to demographic change in these countries. According to WHO estimates, the number of stroke incidents in the EU will presumably increase from 1.1 million per year in 2000 to >1.5 million per year in 2025, solely due to an aging population [4]. Despite extensive resources devoted to rehabilitative and after care, many stroke survivors remain with

residual functional deficits, leaving 30% of patients unable to walk without assistance and 50% suffering from some hemiparesis [5].

While skeletal muscle is the main effector organ accountable for disability in stroke, its importance for short- and long-term outcome is still poorly evaluated. Traditionally, the motor impairment observed in stroke survivors is viewed to result exclusively from the incurred brain injury itself. Structural, metabolic and functional aspects of muscle tissue pathology accompanying stroke are rather episodically addressed.

Indeed, brain injury is the primary cause of disability in stroke. However, hemiparetic apoplexy leads to various muscle phenotype abnormalities such as loss of motoneurons, disuse atrophy and spasticity, adjacent re-innervation, capillary rarefaction and slow-to-fast muscle fiber type shift contrasting that of normal aging, [6–8] These structural adaptive alterations are secondary. They depend considerably on complex pathophysiological reactions including imbalanced efferent neurovegetative signalling, systemic and local metabolic imbalance,

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malnutrition and inflammation [9]. It has been shown that long-term muscle changes not only occur in the paretic but also in the non-paretic limb [10]. It might be reasonably to assume that the changes in muscle morphology may in turn affect function and metabolism.

Systemic pathophysiological pathways following stroke, especially with respect to muscular metabolic integrity, remain only partially understood. To examine these peripheral implications on tissue level, we studied skeletal muscle metabolic activity in patients who suffer from hemiparesis and frailty after apoplexy. **We hypothesize that their muscular metabolic flexibility is limited and more distinct changes can be found in the paretic muscle. Findings in stroke patients were compared to healthy subjects of similar age and BMI.** Microdialysis was applied that allows dynamic *in situ* measurements of muscle intermediate metabolites on tissue level. To investigate possible lateral differences, this minimal-invasive technique was performed on both legs of each patient. Serum levels of marker metabolites and systemic energy expenditure were taken into account. All measurements were performed at rest after overnight fasting and during an oral glucose challenge. This study aims to complement brain- and neuro-specific perspectives to achieve an integrated perception of patients with stroke to advance post stroke physical recovery.

2. Methods

2.1. Study design and population

This was a cross-sectional study with pilot character. *Key inclusion criteria* were stable neurological deficits after completion of all conventional physical therapy at least 6 months after stroke. *Key exclusion criteria* were insulin-dependent diabetes mellitus, primary or secondary myopathies, acute and chronic inflammatory diseases, specific diets, indirect anticoagulation, immunosuppressive therapy or a history of cancer shorter than 5 years. The study protocol was approved by the Institutional Review Board of Charité Medical School Berlin. Written informed consent was obtained from all subjects before study entry.

2.2. Study protocol

All metabolic assessments were performed under standardised conditions, starting in the morning, after an overnight fast (≥ 12 h) and a resting period of at least 20 min in supine position in an air-conditioned and quite room (metabolic ward).

Skeletal muscle tissue metabolism was assessed directly by using microdialysis to monitor interstitial marker metabolites (glucose for substrate supply, lactate and pyruvate for glucose metabolism, and glycerol for lipid mobilisation) of both the paretic and the non-paretic limb. In order to assess also the dynamics in systemic metabolism, blood samples were taken and changes in energy metabolism were monitored by indirect calorimetry. In brief, a catheter (Vasofix® Safety, 20 G; B. Braun, Melsungen, Germany) was placed in a large antecubital vein for blood sampling. One microdialysis probe (M 71, high cut-off of 100,000 kDa, μ Dialysis AB, Solna, Sweden) each was inserted into the *vastus lateralis* muscle. After probe insertion, tissue perfusion was started with lactate-free hydroxyethyl starch solution (+ 50 mmol/L ethanol) at a flow rate of 2 μ L/min, using a M 107 microdialysis pump. Ethanol was added to assess changes in tissue perfusion by using the ethanol dilution technique based on Fick's principle [11]. Accordingly, a decrease in the ethanol outflow-to-inflow ratio (ethanol ratio) is equivalent to an increase in blood flow, and vice versa [11]. After instrumentation, a 60 min period was allowed for tissue recovery from insertional trauma and for baseline calibration. After collection of baseline samples, an oral 75-g glucose load (oGL) was given (300 mL solution, ACCU-CHEK® Dextro®, O.G.T., Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany). Blood and dialysis samples were taken at baseline and every 15 or 30 min after the meal. Carbon dioxide (VCO_2) production and oxygen (VO_2) consumption were measured by a canopy

calorimeter (DeltatracII, Datex Ohmeda, Duisburg, Germany) in order to assess changes in energy expenditure (EE), respiratory quotient ($\text{RQ} = \text{VCO}_2/\text{VO}_2$), and carbohydrate oxidation (CHO) and fatty acid oxidation (FAO) rates according to Ferrannini [12].

2.3. Anthropometrics and functional assessments

Body weight was determined by a weighing scale under standardised conditions (light clothes, no shoes). The BMI was calculated as the ratio of weight (kg) and squared height (m^2). Fat mass (FM) and fat-free mass (FFM) were determined by bioelectrical impedance analysis (BIA) (Bodystat Quadscan 4000, Bodystat, Douglas, Isle of Man, UK). We measured the circumference of both thighs to detect discrepancies in femoral muscle mass as a possible sign of muscle wasting in the paretic vs. non-paretic leg. A functional assessment of femoral muscle isometric strength was realised by knee extension leg test to observe impaired functional capacity in the affected leg.

2.4. Analytical methods and calculations

All blood samples were processed immediately in a refrigerated centrifuge at -4°C , aliquoted and serum and plasma was stored at -80°C until analysis. Blood glucose and insulin concentrations, as well as routine parameters were measured according to international standards. Plasma non-esterified fatty acids (FFA) levels were assayed with an enzymatic method (Wako kit; Unipath, Dardilly, France). Individual insulin sensitivity was assessed by the homeostasis model assessment (HOMA) index as described previously [13,14]. A HOMA value (fasting serum insulin [$\mu\text{U/mL}$] $\times$ fasting plasma glucose [mmol/L]/22.5) of 1 corresponds to normal insulin sensitivity, whereas values >2 indicate the presence of insulin resistance. Ethanol concentrations in the perfusate (inflow) and dialysate (outflow) were measured with a standard spectrophotometric enzymatic assay. Dialysate concentrations of glucose, lactate, pyruvate and glycerol were measured with an automated analyser based on colorimetric principles (ISCUS^{flex}, μ Dialysis, Solna, Sweden). *In situ* dialysate recovery for above metabolites was about 50%, as assessed by near-equilibrium dialysis [15].

2.5. Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD) for group comparison (Table 1) and as mean $\pm$ standard error of the mean (SEM) for repeated measurements after oGL (Figs. 1, 2, 3 and 4). For statistical analysis, standard statistical software packages were used (Statview 5.0; SAS Institute, Cary, N.C., USA and InStat, Version 4.0; Graphpad

Table 1

Group comparison between patients and healthy controls.

Variable	Patients (n = 9)	Controls (n = 8)	P-value
Age (years)	62 $\pm$ 8	58 $\pm$ 5	NS
Men/women (N)	9/0	8/0	NS
BMI (kg/m^2)	28 $\pm$ 4	32 $\pm$ 1	NS
FM (%)	26.7 $\pm$ 4.1	25.0 $\pm$ 5.0	NS
FFM (%)	73.3 $\pm$ 4.1	75.0 $\pm$ 5.0	NS
Ischaemic/haemorrhagic aetiology (N)	8/1	–	
Hemiparesis (%)	100	–	
Fasting glucose (mmol/L)	5.8 $\pm$ 0.7	6.0 $\pm$ 1.4	NS
Fasting insulin ($\mu\text{U/mL}$)	11.8 $\pm$ 4.7	11.8 $\pm$ 4.8	NS
HOMA	3.1 $\pm$ 1.4	3.0 $\pm$ 1.0	NS
HbA1c	5.9 $\pm$ 0.5	5.9 $\pm$ 0.6	NS
Fasting FFA (mmol/L)	0.63 $\pm$ 0.23	0.29 $\pm$ 0.17	0.004

Data are given as mean $\pm$ SD. The non-parametric *U* test for unpaired samples was used for comparing baseline characteristics of stroke patients and healthy controls. BMI, body mass index; FM, fat mass; FFM, fat-free mass; FFA, free fatty acid; HOMA, homeostasis model assessment; NS, non-significant.

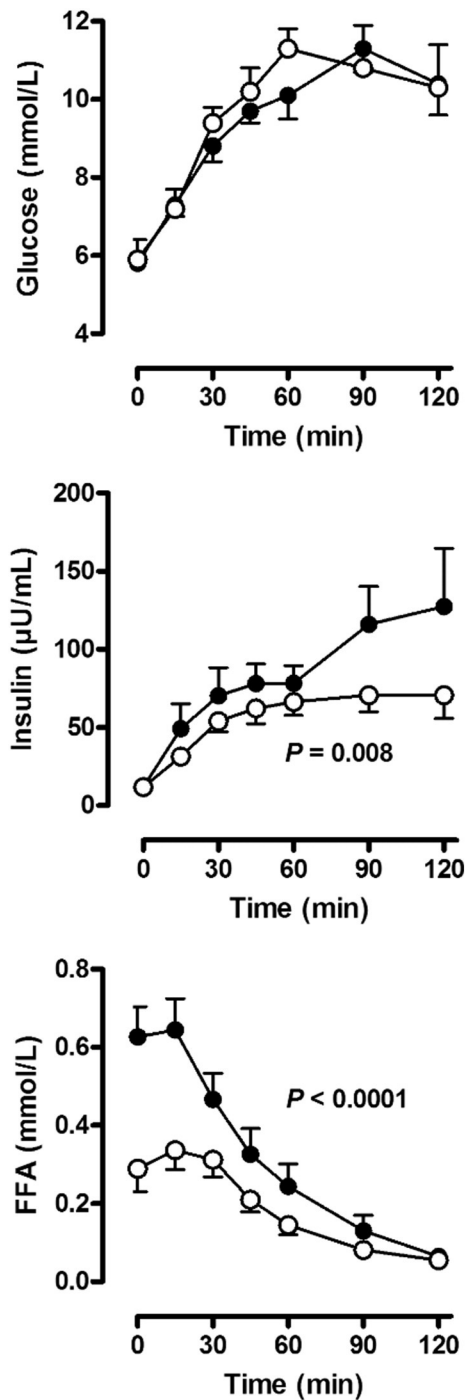


Fig. 1. Oral glucose tolerance test (oGTT). Serum glucose (top), insulin (middle), and free fatty acids (FFA, bottom) in nine hemiparetic stroke patients (closed circles) and eight controls (CTRL, open circles) before and during an oral glucose tolerance test. *P*-values by repeated measures (mixed model) ANOVA.

Software Inc., San Diego, CA, USA). *P*-values < 0.05 were considered to indicate statistical significance. The non-parametric Mann-Whitney *U* test for unpaired samples was used for comparing baseline characteristics of stroke patients and healthy controls in Table 1. Mixed-design ANOVA was used for repeated measurements to compare the response curves for plasma glucose, insulin and FFA and for EE, RQ, as well as skeletal muscle tissue dialysate concentrations of glucose, lactate, pyruvate and glycerol between patients and healthy controls after the oral glucose load.

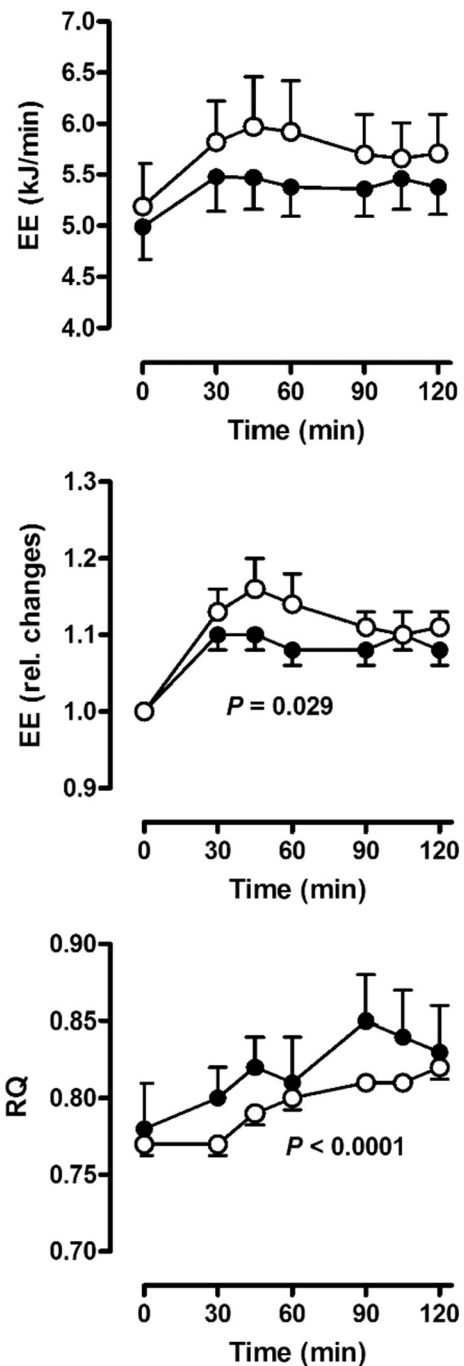


Fig. 2. Substrate oxidation measured by indirect calorimetry. Absolute and relative changes of energy expenditure (EE, top and middle) and respiratory quotient (RQ, bottom) in six hemiparetic stroke patients (closed circles) and seven controls (CTRL, open circles) before and during oral glucose tolerance test. *P*-values by repeated measures (mixed model) ANOVA.

3. Results

3.1. Patients and clinical characteristics

We could enrol nine male patients with hemiparesis of the lower limb after apoplectic ischaemic or haemorrhagic stroke. Patients showed gait asymmetry and/or were dependent on assistive devices such as walker or wheel chair. Eight healthy male volunteers of similar age, BMI and body composition served as controls. Clinical characteristics of patients and healthy volunteers are presented in Table 1.

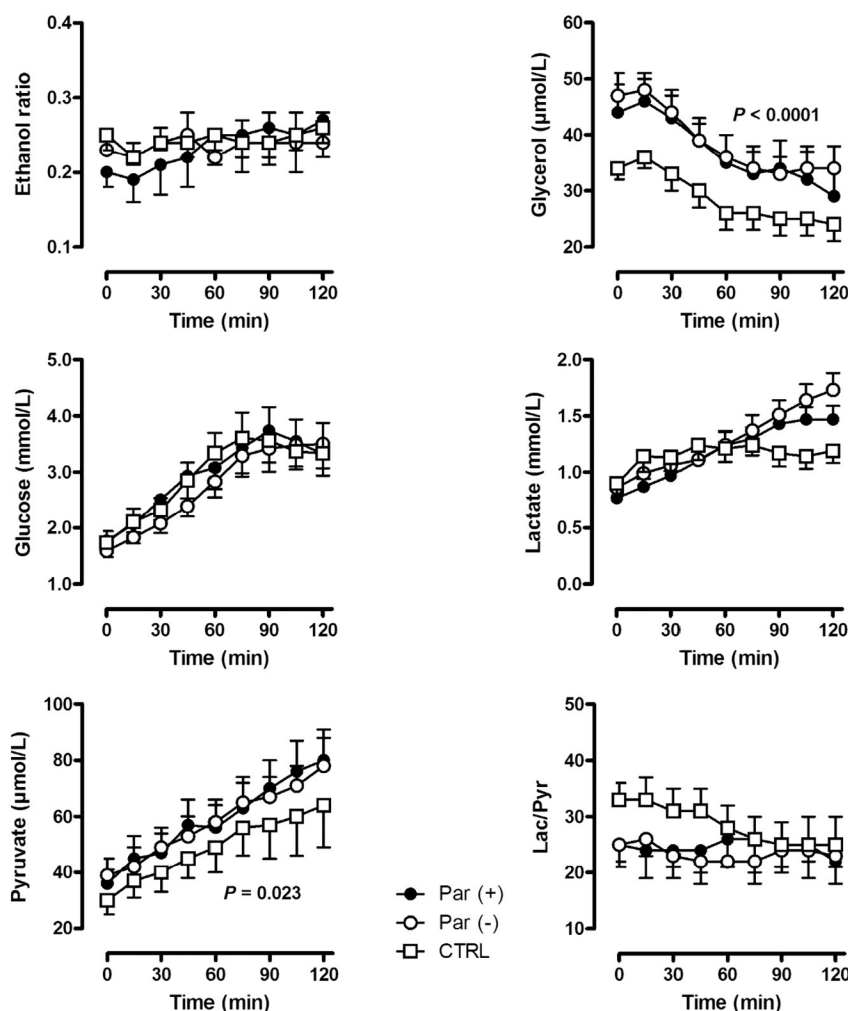


Fig. 3. Skeletal muscle microdialysis. Skeletal muscle microdialysis. Ethanol ratio and dialysate concentrations of glucose, lactate, pyruvate and glycerol and lactate/pyruvate (Lac/Pyr) ratio in skeletal muscle of the paretic (Par +) and non-paretic (Par -) leg in nine hemiparetic stroke patients and eight controls (CTRL). P-values Par (+) vs. CTRL by repeated measures (mixed model) ANOVA.

Three stroke patients required assistive devices (1 wheel chair, 2 single-point canes). Median stroke latency was 23 months, ranging from 7 to 34 months poststroke. Maximum isometric muscle strength of the impaired extremity was 20% lower compared to the non-affected leg (348 ± 64 vs. 433 ± 117 N), but this did not reach significance ($P = 0.15$). Same applies to bilateral femoral circumference (paretic; 53.2 ± 3.7 cm vs. non-paretic; 54.2 ± 4.0 cm; $P = 0.41$).

3.2. Blood parameter

Postprandial dynamic profiles of glucose, insulin and FFA are shown in Fig. 1. Baseline plasma concentrations of glucose and insulin did not differ significantly between patients and healthy subjects (Table 1). Plasma glucose concentrations increased twofold in both groups. At 2 h after oral glucose challenge, the plasma glucose concentration was 10.4 ± 0.8 mmol/L for patients and 10.3 ± 1.1 mmol/L for controls, respectively (Fig. 1, top). Insulin concentrations rose 11-fold in patients and 6-fold in controls, reaching maximum values of 127.5 ± 37.3 μ U/mL and 70.7 ± 10.7 μ U/mL, respectively (Fig. 1, middle). FFA levels were significantly higher in stroke patients at baseline. The FFA levels decreased by ~90% in patients and by 80% in controls (to 0.07 ± 0.02 vs. 0.06 ± 0.01 mmol/L; Fig. 1, bottom). Insulin sensitivity was likewise reduced in stroke patients and controls. Therefore significance was not reached (Table 1).

3.3. Systemic energy expenditure and substrate utilisation

Resting energy expenditure was not different in patients vs. controls (Fig. 2, top). However, relative postprandial increase after glucose stimulation was lessened in patients, compared to controls ($P = 0.029$; Fig. 2, middle).

Resting RQ was similar in patients vs. controls. After the glucose load, postprandial RQ increased in both groups, however, in patients faster and also at a significantly higher level, compared to healthy controls ($P < 0.001$; Fig. 2, bottom).

4. Skeletal muscle metabolism

4.1. Tissue perfusion

Baseline skeletal muscle tissue ethanol ratio, and hence tissue perfusion, did not differ significantly between paretic or non-paretic lower limbs. Both groups did not show significant changes in tissue perfusion during oral glucose tolerance testing (oGTT) (Fig. 3, upper left graph).

4.2. Marker metabolites

Baseline muscle dialysate glycerol values did not differ significantly between the paretic and non-paretic leg. Fasting and postprandial

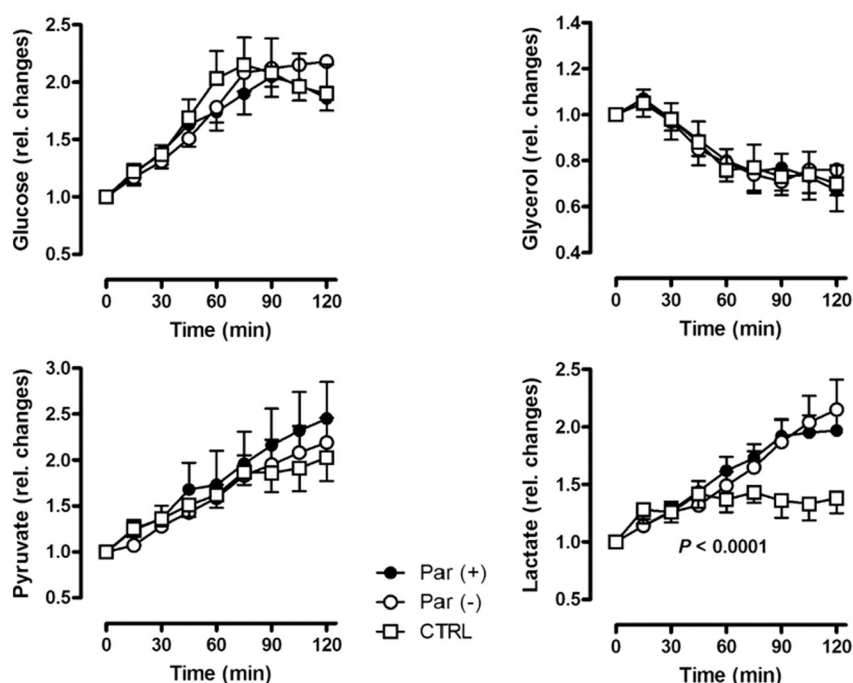


Fig. 4. Skeletal muscle microdialysis (relative changes). Dialysate concentrations of glucose, lactate, pyruvate and glycerol (relative changes) in skeletal muscle of the paretic (Par +) and non-paretic (Par –) leg in nine hemiparetic stroke patients and eight controls (CTRL). *P*-values Par (+) vs. CTRL by repeated measures (mixed model) ANOVA.

dialysate glycerol levels were significantly higher in patients vs. controls ($P < 0.001$; Fig. 3, upper right graph). Postprandial dialysate glycerol decreased altogether by approximately 25% in patients and 30% in controls, without showing any disparity in postprandial regression (Fig. 4, upper right graph).

There were no differences in preprandial dialysate glucose values neither between both limbs in stroke patients nor between patients and healthy controls. After the oGL, dialysate glucose increased approximately twofold within 75 min (parallel to plasma glucose levels), and remained at that level until the end of the test without significant differences on both counts (Fig. 3, middle left graph; Fig. 4, upper left graph).

Dialysate lactate values, again, showed no significant difference between paretic and non-paretic leg in patients at baseline and after glucose administration. Comparing paretic to control muscle, no difference could be found in dialysate lactate values, yet (Fig. 3, middle right graph). In regard to the non-paretic muscle, however, response rates of dialysate lactate were significantly higher in comparison to control muscle ($P = 0.03$). Also, the relative increase in dialysate lactate levels after oral glucose challenge was generally higher in patients vs. controls ($P < 0.001$). In patients, postprandial dialysate lactate doubled showing almost linear increase, whereas lactate values for controls increased moderately within the first 45 min of oGTT and remained at this plateau throughout the rest of the test (Fig. 4, lower right graph).

Dialysate pyruvate showed no discrepancy between both legs in stroke patients. Dialysate pyruvate was significantly higher in patients at baseline and during the oGTT in comparison to the control group ($P = 0.023$ for paretic vs. control muscle; $P = 0.026$ for non-paretic vs. control muscle; Fig. 3, lower left graph). Pyruvate values increased steadily and eventually twofold throughout postprandial testing. There were noticeable fluctuations in dialysate pyruvate data for the impaired limb which increased 2.5-fold over the course of the test, but this did not reach the level of significance (Fig. 4, lower left graph).

Lactate/pyruvate (Lac/Pyr) ratio of patients showed no significant difference between paretic and non-paretic skeletal muscle. Whereas the Lac-Pyr ratio remained overall at a low, stable level in patients, it displayed a rather sigmoidal trend in controls. Control group values began to decrease 15 min after glucose challenge and dropped to a

lower, steady level after 75 min, which was similar to that of patients (Fig. 3; lower right graph). This relation between lactate and pyruvate did not reach significance in paretic compared to control muscle. It was, however, significantly different between non-paretic and control muscle ($P = 0.001$). Thereby Lac/Pyr ratio illustrated a trend towards glycolytic activity in poststroke skeletal muscle, as likewise indicated by postprandial dynamics in lactate values of poststroke skeletal muscle.

4.3. Discussion

This is the first study to provide *in situ* investigation of musculoskeletal metabolism in the poststroke phase. We tested the hypothesis that muscular metabolic capacity is limited in patients after hemiparetic stroke, and that changes affect rather the contralateral (impaired) than the ipsilateral (non-impaired) limb. In this study, we found no differences in skeletal muscle tissue metabolism between paretic and non-paretic leg in patients with stroke. However, patients showed impaired substrate metabolism compared to healthy controls with

- increased lactate and glycerol production on tissue level;
- delayed and impaired glucose utilisation;
- reduced increase in postprandial energy expenditure.

Plasma glucose profiles did not differ significantly between patients and controls. In both groups, plasma glucose levels > 7.8 mmol/L were found 2 h after the oGL, indicating an impaired glucose tolerance in both groups [16]. Consistent with this finding, the majority of patients exhibited reduced insulin sensitivity. Anomalous glucose metabolism is highly prevalent in stroke patients [17], consequently promoting cardiovascular disease risk and deconditioning. Although we could not demonstrate significance in comparison to healthy controls, the clinical relevance of this finding is thereby not attenuated. The mean BMI of our control group suggests moderate obesity according to WHO classification [18]. There is also a wide recognition that reduced insulin sensitivity is strongly linked to obesity [19,20].

In order to eliminate possible modifications of our results due to altered transcapillary supply [21], we determined tissue ethanol ratio over the entire analysis, as illustrated above (see Section 2.2). As shown from the ethanol ratio in the microdialysis study, capillary perfusion was similar in stroke patients and controls at fasting and after oral glucose challenge. Without haemodynamic changes, fluctuations in interstitial concentration of marker metabolites cannot be attributed to modified substrate delivery or product clearance. Therefore, they may reflect true metabolic changes in muscle after physiological glucose challenge.

Our findings imply a normal glucose delivery and uptake in skeletal muscle tissue after the oGL. Tissue dialysate glucose profiles did not show significant differences between patients and controls. We measured pyruvate as an indicator of changes in the aerobic oxidative route. In muscle, pyruvate is mainly oxidized to acetyl-CoA and CO₂ to fuel the citric acid cycle during cellular respiration. Alternatively, lactate is produced from pyruvate as a less sufficient but supplementary source of energy. It functions as an indicator of changes in the anaerobic glycolytic route regarding overall glucose turnover in skeletal muscle. Although dialysate pyruvate concentrations were higher in stroke patients than controls, we could demonstrate a pronounced increase in lactate values in bilateral skeletal muscle during oral glucose tolerance testing. This trend was supported by higher lactate values and a significant correlation in response rates of interstitial lactate and pyruvate concentrations in non-paretic compared to control muscle. Our findings, therefore, indicate an enhanced activity of the glycolytic route in poststroke skeletal muscle.

In stroke patients, we also observed increased baseline and postprandial muscle tissue interstitial glycerol concentrations, indicating an activated lipolytic activity on tissue level [22]. This was accompanied by higher systemic concentrations in FFAs compared to healthy subjects. In patients and controls, plasma FFA levels exhibited a distinct drop upon oral glucose challenge. As we observed a stronger increase in insulin concentrations after glucose loading in patients, the decrease in FFA levels was even more pronounced in these subjects. This was mirrored by a decrease in glycerol concentrations on tissue level in both groups. The reduction in lipolytic metabolites is likely based on the anti-lipolytic effect of insulin, although insulin resistance was present in patient and control group. It also reflects the physiological response to increased glucose utilisation as the predominant substrate after oral glucose intake. This effect was supported by postprandial RQ values.

In patients, RQ indicated an almost equally mixed carbohydrate and fat utilisation after overnight fast; whereas carbohydrate oxidation rate was lower in controls. This could be either consequence of greater glycogen stores or result more likely from a blunted resting metabolic rate with reduced lipid mobilisation and oxidation. In patients, RQ began to increase immediately after glucose load and reached higher values compared to controls. The immediate increase in RQ, and consecutively carbohydrate utilisation after glucose loading, argues against impaired metabolic flexibility due to mitochondrial dysfunction. We propose that the impaired response rate of EE after glucose loading may result from a reduction in ambulatory physical activity patterns, which is a frequent observation after hemiparetic stroke [23].

Several adaptations in skeletal muscle morphology after stroke have been described previously [24]. Those include diverse effects on paretic vs. non-paretic muscle, but also systemic effects on muscle tissue [25, 26]. *Vastus lateralis* muscle is naturally considered as a mixed muscle with equal composition of fast (types IIa or IIx) and slow (type I) myosin heavy chains (MHC) which present different metabolic properties [27]. Recently, intra-individual differences in muscle fiber profile have been identified in hemiparetic stroke survivors. These differences displayed a significant shift from oxidative fibers of type I towards glycolytic fibers of type IIx MHC in paretic *vastus lateralis* muscle [28]. De Deyne PG et al. [23] demonstrated an inverse association between the increase in fast type MHC fibers in affected vs. non-affected muscle

and the self-selected gait speed. This was not accompanied by altered metabolic activity of glycolytic or oxidative enzymes. In a more recent study, Severinsen K et al. [29] reported a phenotypic shift towards more fatigable muscle fibers, as well as reduced oxidative enzymatic capacity in paretic muscle. This correlated with aerobic capacity. Contrarily, no fiber-type transformation could be found in 16 ambulatory stroke patients, of which 15 could walk without assistance [30]. We conclude that changes may only be observed in more pronounced impairment. However, functional disability from stroke often promotes physical inactivity [21]. Inactivity itself likewise induces a decline in slow-twitch and a corresponding increase in fast-twitch MHC isoforms [24,31].

In the present study, we could not detect a disproportionate metabolic function between both *vastus lateralis* muscles, but observed an enhancement in overall lipolytic and postprandial glycolytic activity. This could suggest an underlying fiber-type shift towards the predominantly fast glycolytic type II MHC isoforms. It is assumed that fast MHC isoforms are also more resistant to insulin than slow isoforms, [32,33] Consequently, fast isoforms are rather characterised by reduced oxidative capacity with impaired glucose oxidation and therefore greater lipolytic and glycolytic activity. Partly conflicting data regarding metabolic function of poststroke muscle could be explained by heterogeneity among study populations between our study and previous reports. This heterogeneity regards severity of disablement (indicated by requirement of assistive devices) and mean stroke latency. We assume that muscular alterations may potentially be more distinct in severely disabled stroke survivors and that changes might become more apparent over time. Patients with greater neurological deficit may be prone to greater disuse. Systemic muscle cell alteration after stroke has been reported previously as indicated by elevated levels of CAF22, a potential marker of neuromuscular integrity and re-structuration after injury [34]. Further studies are needed to determine whether stroke-specific tissue alterations or secondary disuse as a more general consequence of stroke account for these results.

Our study has, of course, some limitations. The relatively small sample size may prevent identification of significant differences between groups and of differences between the paretic vs. non-paretic leg. With a greater sample size, greater neurological deficit and latency to cerebral infarction, statistical significance could possibly be met, especially concerning the anticipated alterations in paretic muscle properties.

Our study excels at correlating the *in-situ* tissue metabolic measurements with whole body plasma measurements and, particularly, with systemic energy expenditure studies. Using microdialysis methodology allows for investigation of tissue metabolic processes beyond the intravascular compartment. Therefore, it avoids the higher risk and strain to patients of whole tissue samples by biopsies. The use of bilateral muscle sampling eliminates individual variation in terms of diet, habitual activity level or genetics. Future research might investigate patients with greater neurological deficit severity and focus on studying muscle metabolic performance during moderate or extensive exercise. We believe that our study provides a promising novel approach to examine metabolic alterations in poststroke skeletal muscle on tissue level.

5. Conclusion

This study provides novel insights into the metabolic changes of skeletal muscle tissue in the poststroke phase. In summary, the current study shows no difference in skeletal muscle metabolic properties between the paretic and non-paretic leg of hemiparetic stroke subjects. Our findings imply an impaired substrate metabolism in bilateral skeletal muscle in comparison to healthy subjects. An increase in glycolytic and lipolytic activity in poststroke skeletal muscle suggests reduced oxidative capacity and might indicate a fiber-type shift. Further research is needed to study secondary implications of stroke on tissue level that will benefit an integrated perception of stroke patients and poststroke muscular integrity.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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