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

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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/m^2; 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.

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 rarefication 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, μDialysis 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 μL/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 ($RQ = VCO_2/VO_2$), 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 [μU/mL] x 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}, μDialysis, 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 ± standard deviation (SD) for group comparison (Table 1) and as mean ± 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 ± 8	58 ± 5	NS
Men/women (N)	9/0	8/0	NS
BMI (kg/m^2)	28 ± 4	32 ± 1	NS
FM (%)	26.7 ± 4.1	25.0 ± 5.0	NS
FFM (%)	73.3 ± 4.1	75.0 ± 5.0	NS
Ischaemic/haemorrhagic aetiology (N)	8/1	–	
Hemiparesis (%)	100	–	
Fasting glucose (mmol/L)	5.8 ± 0.7	6.0 ± 1.4	NS
Fasting insulin (μU/mL)	11.8 ± 4.7	11.8 ± 4.8	NS
HOMA	3.1 ± 1.4	3.0 ± 1.0	NS
HbA1c	5.9 ± 0.5	5.9 ± 0.6	NS
Fasting FFA (mmol/L)	0.63 ± 0.23	0.29 ± 0.17	0.004

Data are given as mean ± 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.

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