Resistive Training and Molecular Regulators of Vascular-Metabolic Risk in Chronic Stroke

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Background: Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC-1α) gene and Sirtuin-1 (SIRT-1) respond to physiological stimuli and regulate insulin resistance. Inflammatory markers tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), and the soluble forms of intracellular adhesion molecule (sICAM-1) and vascular CAM-1 (sVCAM-1) are associated with increased risk of diabetes and coronary heart disease. Resistive training (RT) reduces hyperinsulinemia and improves insulin action in chronic stroke. Yet, the molecular mechanisms for this are unknown. This study will determine the effects of RT on skeletal muscle PGC-1α and SIRT-1 mRNA expression and inflammatory and vascular markers. *Methods:* Stroke survivors (50-76 years) underwent a fasting blood draw for measurement of TNF-α, IL-6, CRP, serum amyloid A, sICAM-1, sVCAM-1, and bilateral vastus lateralis biopsies before and after RT. Participants were also assessed using bilateral multislice thigh computed tomography scans from the knee to the hip, a total body scan by dual-energy X-ray absorptiometry, and 1-repetition maximum strength testing. Subjects performed 2 sets of 3 lower extremity RT exercises 3 times per week for 12 weeks. *Results:* Bilateral leg press and leg extension strength increased ~30-50% with RT ($P < .001$). Body weight, total body fat mass, and fat-free mass did not change. Thigh muscle area and volume increased in both legs ($P < .05$). Nonparetic muscle PGC-1α mRNA expression increased 14% ($P < .05$) after RT and SIRT-1 mRNA decreased 24% ($P < .05$) and 31% ($P < .01$) in paretic and nonparetic muscles. There were no significant changes in plasma inflammation with training. *Discussion:* RT in chronic stroke induces changes in key skeletal muscle regulators of metabolism, without effecting circulating inflammation. **Key Words:** Exercise—skeletal muscle—stroke—inflammation—strength—vascular.
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Introduction

Inflammation is a risk factor for stroke and contributes to the progression of cardiovascular disease.[1] Moreover, low-grade inflammation is a pathophysiological mechanism underlying sarcopenia.[2] The paretic thigh of stroke survivors has 20% lower muscle area and 25% higher intramuscular fat than the nonparetic thigh[3] demonstrating substantial atrophy and muscle composition change.[4] We have previously reported that resistive training (RT) results in muscle hypertrophy and loss of intramuscular fat in chronic stroke, while reducing skeletal muscle expression of myostatin,[5] a member of the transforming growth factor beta family of secreted growth factors, and a significant regulator of skeletal muscle development and size.[6] To our knowledge, no studies have examined RT-induced changes to key inflammatory and metabolic biomarkers in the circulation after stroke or their role in paretic muscle wasting.

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C-reactive protein (CRP) and the soluble forms of intracellular adhesion molecule (sICAM-1) and vascular CAM-1 (sVCAM-1) are vascular inflammatory markers associated with increased risk of diabetes and coronary heart disease.[7,8] Elevated circulating concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) also occur in type 2 diabetes and predict its development in middle-aged and elderly adults.[9,10] In addition, peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC-1α) and Sirtuin-1 (SIRT-1) respond to physiological stimuli and regulate insulin resistance though distinct mechanisms.[11] Regular aerobic exercise modulates intracellular pathways to improve glucose uptake, in part by attenuating epigenetic modifications on PGC-1α and its downstream regulators.[12]

We showed that RT can reduce hyperinsulinemia and improve insulin action in chronic stroke,[13] a population with a high prevalence of insulin resistance and diabetes.[14] Herein, we test the hypothesis that RT reduces systemic inflammation and alters the gene expression of PGC-1α and SIRT-1 in paretic and nonparetic skeletal muscles. Thus, the purpose of this study was to determine the effects of RT on systemic inflammatory and vascular markers, and paretic (P) and nonparetic (NP) skeletal muscle PGC-1α and SIRT-1 mRNA expression in chronic stroke.

Methods

Subjects

Of the 24 ischemic stroke subjects enrolled (>6 months latency), 6 individuals did not complete the study because of time constraints or medical issues unrelated to study participation. The 18 individuals (12 men, 6 women) who completed the study were between 55 and 76 years old with body mass indexes between 21 and 39 kg/m^2. All 18 individuals underwent either a blood drawn for assessment of circulating inflammatory markers or bilateral-skeletal muscle tissue biopsies for gene expression analysis. Fifteen individuals came from our previously published study[5] but the blood and tissue biomarkers covered in this paper were not part of the prior work. All stroke survivors had mild to moderate hemiparetic gait deficits and had completed conventional rehabilitation therapy. Evaluations included medical history, physical examination, fasting blood profile, and screening for dementia[15] and depression[16] to ensure adequate informed consent. Subjects were excluded for unstable angina, congestive heart failure (New York Heart Association Class II), severe peripheral arterial disease, major poststroke depression, dementia, severe receptive aphasia, and orthopedic or chronic pain conditions.

All tests were performed before and after the 3-month training intervention. All methods and procedures were approved by the Institutional Review Board of the University of Maryland as well as the VA R&D committee. Each participant provided written informed consent.

VO_2peak and Body Composition

Exercise testing with open circuit spirometry was conducted to measure VO_2peak using a graded submaximal treadmill test.[17] Height and weight were measured. Fat mass, lean tissue mass, and %body fat were determined by dual-energy X-ray absorptiometry (DXA; Prodigy LUNAR GE, version 7.53.002, General Electric Company, Madison, Wisconsin, USA). Thigh computed tomography scans were performed every 4 cm starting at the patella and ending at the femoral head (Siemens Somatom Sensation 64 Scanner, Siemens Co, Cleveland, OH, USA), and a single mid-thigh slice was used to quantify skeletal muscle area, total fat area, low-density lean tissue area,[3] and muscle attenuation in both the paretic and nonparetic thighs. Scans were analyzed using MIPAV (Medical Image Processing, Analysis and Visualization, v.7.0, NIH, National Institutes of Health, Bethesda, MD, USA).

Strength Testing

Bilateral 1-repetition maximum (1-RM) strength tests were conducted on pneumatic leg press and leg extension RT equipment built for single leg movement (Keiser, Fresno, CA), to account for strength discrepancies between the paretic and nonparetic limbs. Two familiarization sessions were included prior to baseline 1-RM testing to avoid the confounding effects of learning on baseline strength measures.

Blood Draw and Analysis

Subjects underwent an overnight 12-hour fast and on the following morning had a blood draw (n = 15) and 2-hour oral glucose tolerance test. Blood samples were collected in heparinized syringes, placed in prechilled test tubes containing 1.5 mg of ethylenediaminetetraacetic acid per milliliter of blood, centrifuged at 2000× *g* for 10 minutes at 4°C, and aliquoted for storage at –80°C until analysis. Plasma glucose concentrations were measured in duplicate using the glucose oxidase method (2300-STAT Plus; YSI, Yellow Springs, OH). Plasma insulin was determined in duplicate using radioimmunoassay (Millipore, Billerica, MA). Fasting plasma for CRP, serum amyloid A (SAA), sICAM-1, and sVCAM-1 was measured in duplicate, with coefficient of variation (CV) less than 10% according to electrochemiluminescence using a multispot microplate (SECTOR Imager-2400; Meso Scale Discovery, Gaithersburg, MD). Fasting plasma for TNF-α, IL-6, IL-1β, and IL-8 was measured in triplicate also using a multispot microplate (SECTOR Imager-2400, Meso Scale Discovery).

Skeletal Muscle Biopsies, RNA Extraction, and Reverse Transcription for Real-Time RT-*PCR*

Percutaneous needle biopsies were obtained from the *vastus lateralis* muscle. These were done ~12-13 cm above the patella on the anterolateral aspect of each thigh using a Bergstrom needle (Stille, Solna, Sweden) with a