Elevated Tumor Necrosis Factor-α in Skeletal Muscle After Stroke

Charlene E. Hafer-Macko, MD; Shuzhen Yu, MD; Alice S. Ryan, PhD; Frederick M. Ivey, PhD; Richard F. Macko, MD

Background and Purpose—Tumor necrosis factor-α (TNF-α), an inflammatory cytokine negligibly expressed in normal muscle, is elevated in selected metabolic conditions characterized by muscle wasting and insulin resistance. Inflammation is fundamental to stroke pathogenesis. Stroke patients have gross muscular atrophy and high prevalence of diabetes and insulin resistance. Yet, no previous studies examined TNF-α expression in hemiparetic skeletal muscle. This study investigates whether TNF-α mRNA levels are elevated in paretic compared with nonparetic leg muscles of chronic ischemic stroke patients and age-matched controls.

Method—Total RNA extracted from bilateral vastus lateralis muscle biopsies from n=20 hemiparetic stroke patients and n=9 healthy controls was reverse transcribed to cDNA, then TNF-α transcripts were amplified by real-time quantitative polymerase chain reaction. TNF-α mRNA concentrations were normalized against acidic ribosomal phosphoprotein, a housekeeping gene.

Results—TNF-α mRNA levels were 2.8-fold higher in paretic compared with control leg muscle (6.28±1.86 versus 2.28±0.67; P<0.03) and 1.6-fold higher in nonparetic leg (3.71±1.02; P<0.11) compared with controls. There was a trend for higher TNF-α mRNA in paretic compared with nonparetic leg.

Conclusions—Findings demonstrate increased TNF-α expression in paretic leg muscle, suggesting inflammatory pathways are accelerated in stroke muscle. Further studies are under way to determine whether intramuscular inflammatory pathways contribute to atrophy and metabolic abnormalities after stroke. (Stroke. 2005;36:2021-2023.)

Key Words: inflammation ■ insulin ■ stroke ■ tumor necrosis factor

Inflammation is a fundamental pathogenic mechanism in atherosclerosis and stroke.1,2 Elevated inflammatory markers predict risk for incident and recurrent stroke.3,4 Tumor necrosis factor-α (TNF-α), a pivotal cytokine, upregulates systemic inflammatory markers, mediates insulin resistance, and increases cardiovascular risk.5,6 Associations between type 2 diabetes and muscle TNF-α overexpression are strong and are attributed to TNF-α actions on muscle insulin signaling.6 Stroke survivors have a 70% prevalence of diabetes and insulin resistance.9 Despite evidence linking inflammation with stroke risk, diabetes, and insulin resistance, the tissue source(s) for cytokines have not been systematically investigated in the stroke population.

Stroke is a leading cause of chronic disability, possibly attributable to secondary abnormalities in skeletal muscle.8,10 We report gross muscular atrophy and phenotype shift in hemiparetic thigh that independently predict poor fitness levels and gait severity after stroke.8,10,11 Although skeletal muscle TNF-α can mediate muscular wasting and physical frailty in advancing age,7 no previous studies investigated TNF-α expression in hemiparetic muscle after stroke. The hypothesis of this cross-sectional study is that TNF-α mRNA expression is elevated in paretic compared with nonparetic leg muscle after stroke. Because stroke may constitute a systemic biological process, muscle biopsies from healthy age-matched sedentary community reference controls are examined to determine whether hemiparetic leg muscle TNF-α is abnormal.

Patients and Methods

Men and women ≥45 years of age with hemiparetic gait after remote (>6 months) ischemic stroke underwent medical history, examinations, and timed 30-foot walks. Exclusion criteria included conditions limiting mobility, anticoagulation, and recent (<3 weeks) infection or inflammation. Individuals providing written informed consent had bilateral vastus lateralis (VL) muscle biopsies.8 VL muscle was also obtained from age- and body mass index (BMI)—matched healthy community controls without neurological disease or gait impairment before initiating research studies of diet and exercise. The University of Maryland institutional review board approved all aspects of this study.
### Clinical and Demographic Data

<table>
<thead>
<tr>
<th></th>
<th>Stroke n=20</th>
<th>Controls n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66±8</td>
<td>65±8</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>17:3*</td>
<td>5:4</td>
</tr>
<tr>
<td>BMI</td>
<td>30±7</td>
<td>31±4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (75%)</td>
<td>5 (55%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>16 (80%)*</td>
<td>4 (44%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (47%)*</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>4 (21%)</td>
<td>4 (44%)*</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD or n (%). *P<0.05, difference between stroke and control.

### Analyses

Paired and unpaired t tests examined significance of differences in TNF-α levels between paretic versus nonparetic stroke muscle and versus controls. Data were presented as mean±SE. Significance was set at P<0.05. χ² determined whether there were differences in proportions of cardiovascular disease risk factors between stroke and controls.

### Results

The Table presents clinical and demographics features of 20 stroke patients and 9 healthy control subjects. Timed 30-foot walking speed for stroke patients was 1.16±0.15 miles per hour. Stroke latency was 7 to 108 months. TNF-α mRNA levels were significantly higher in stroke paretic thigh compared with controls (6.28±1.86 versus 2.28±0.67; P<0.03). There was a trend toward elevated TNF-α in paretic compared with nonparetic thigh (Figure; 3.71±1.02; P=0.1). Hence, skeletal muscle TNF-α mRNA levels were 2.8-fold higher in paretic and 1.6-fold higher in nonparetic VL muscle compared with controls. Regression analyses further revealed a significant relationship between TNF-α transcript in paretic and nonparetic thighs (r=0.52; P=0.02). There were no significant relationships between TNF-α mRNA level with age, gender, BMI, stroke latency, walking speed, or presence of hypertension, dyslipidemia, diabetes, or impaired glucose tolerance.

### Discussion

We demonstrate elevated TNF-α transcript in thigh muscles after stroke; this elevation is not confined to paretic leg but is present bilaterally. TNF-α is normally negligible in human skeletal muscle but is elevated in several metabolic conditions. Our 2.8-fold increased TNF-α expression in paretic muscle compared with controls is similar in magnitude to the 2- to 4-fold elevation in muscle with diabetes and aging. Surprisingly, we observed a 1.6-fold increased TNF-α level in nonparetic VL muscle compared with controls. This might be attributable to disuse because reduced physical activity is linked to systemic inflammation. The significant positive relationship between TNF-α transcript levels in paretic and nonparetic legs suggests a common mechanism. Although we observed a trend for greater TNF-α expression in paretic leg, bilateral elevation raises the possibility that more diffuse inflammatory pathway activation contributes to systemic inflammatory–metabolic syndromes, in addition to asymmetric local structural–functional abnormalities in muscle after stroke.

Clinical and experimental data implicate an important role for inflammation in the pathogenesis of stroke and disability, which may constitute a modifiable risk factor. The precise tissue location(s) of inflammation after stroke has not been identified. Increased muscle TNF-α could serve as a depot for inflammation predisposing to insulin resistance and muscle atrophy, which are prevalent in stroke populations. Careful pathology studies are planned to confirm cellular source(s) of TNF-α in muscle.

In summary, we report elevated skeletal muscle TNF-α in chronic hemiparetic stroke survivors, raising the possibility that inflammation alters muscle structure and metabolic function to propagate disability. Our results must be interpreted cautiously because small sample size is limited and it focuses exclusively on TNF-α transcript. Other investigators report that TNF-α protein levels and biological activity parallel transcript expression. This study did not examine elevations of TNF-α protein or upstream and downstream inflammatory pathway activation. Further studies are necessary to define the full inflammatory pathway activation profile in muscle after stroke and whether inflammation relates to local muscle abnormalities or systemic metabolic–inflammatory status predicting recurrent stroke risk.

### Acknowledgements

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**Figure 1.** Real-time RT-PCR TNF-α mRNA concentration (mean and SE) normalized against housekeeping gene in paretic (P) and nonparetic (NP) thigh after stroke and control (C) muscles. Paretic thigh TNF-α was significantly elevated compared with control muscles (P<0.05).
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References
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