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Running head: STROKE, RAT HINDLIMB MUSCLE

Effect of Inactivity and Undernutrition following Acute Stroke on Rat Hindlimb
Muscle Mass, Myofibrillar Protein Content and Type I, II Fiber Cross-sectional
Area

Key Words: cerebral infarction, muscle atrophy

1 **Abstract**

2

3 Background: Stroke patients experience functional changes resulting from muscle atrophy
4 related to disuse, lack or limited neuronal stimulation, and undernutrition. Acute stroke is
5 assumed to induce muscle atrophy, however, there is little information regarding muscle changes
6 following acute stroke.

7 Objective: The purpose of this study was to examine the effect of inactivity and undernutrition
8 following acute stroke on mass, myofibrillar protein content and type I, II fiber cross-sectional
9 area of rat hindlimb muscles.

10 Methods: Adult male Sprague-Dawley rats (240 – 270g body weight) were randomly assigned to
11 1 of 3 groups, stroke (n= 7; occlusion of the right middle cerebral artery [RMCA]); control (n=
12 7; sham RMCA procedure); and undernourished (n= 9; pair – fed to match intake of stroke rats).
13 Food and water intake and body weight were measured daily. Seven days post occlusion or sham
14 occlusion, rats were anesthetized and soleus (type I), plantaris (type II) and gastrocnemius (type
15 II) muscles were dissected from both the affected and unaffected sides. The brain was sectioned
16 to identify cerebral infarction in the stroke group. Body weight, food intake, muscle weight, fiber
17 type distribution, cross-sectional area and myofibrillar protein content of dissected muscles were
18 determined.

19 Results: Compared to the control group, seven days following stroke, diet intake and body
20 weight decreased in the stroke group ($p = 0.001$, $p = 0.03$) and concomitant decreases were also
21 noted in pair-fed rats ($p = 0.001$, $p = 0.04$). In the stroke group, compared with the controls, the
22 following changes were noted in both the affected and unaffected limbs: soleus and
23 gastrocnemius muscle weights (less in stroke group, $p = 0.02$, $p = 0.03$); soleus type I fiber cross-
24 sectional area (less in stroke group, $p = 0.03$); soleus myofibrillar protein content (less in stroke
25 group, $p = 0.03$). Changes in the undernourished group were similar in direction to the stroke
26 group, but failed to achieve statistical significance when compared with the control values.

27 Conclusion: Hindlimb muscle atrophy occurs in both affected and unaffected sides following
28 acute stroke, with type I muscle changes more apparent than type II muscle.

29

1 **Introduction**

2 As the average life span has increased so has the incidence of stroke (Sacco, 1995). For
3 example, stroke is the leading cause of death in Korea (Korean Statistical office, 2003). In the
4 United States, stroke is nation's third leading cause of death, killing nearly 160,000 Americans
5 every year (American Speech-Language-Hearing Association, 2003). Stroke is a leading cause of
6 serious, long-term disability in the United States (American Stroke Association, 2003), and one
7 of the important causes of physical disability (Duncan, 1994).

8 With recent advanced medical therapies, the early death rate of stroke is gradually decreasing
9 and the survival rate from stroke is increasing. It is estimated that over 50% of patients survive
10 the initial stroke injury (Samsa, Bian, Lipscomb, & Matchar, 1999). Over 60% of stroke patients
11 lead their lives with persistent limb weakness related to the stroke (Lawrence, Coshall, Dundas,
12 Stewart, Rudd, Howard, & Wolfe, 2001). Optimizing patient outcomes following stroke
13 necessitates an understanding the effects of cerebral ischemia that may impact on the patient's
14 rehabilitation potential. For example, stroke patients often experience paralysis or paresis on the
15 affected side, which may impact walking and the performance of activities of daily living
16 (Schneider & Gautier, 1994). These functional changes could be the result of muscle atrophy
17 related to disuse and lack or limited neurologic stimulation (denervation). Physical inactivity
18 associated with hemiparesis may compound the disuse of skeletal muscle groups (Potempa,

1 Braun, Tinknell, & Popovich, 1996). Additionally, altered nutrition may contribute to structural
2 changes. Understanding muscle changes following cerebral ischemia may provide important
3 insights to maximize functional ability.

4 In humans, muscle fiber size and cross-sectional area of the affected side decreases resulting
5 in muscle atrophy (Scelsi, Lotta, Lommi & Marchett, 1984; Chokroverty, Reyes, Rubino, &
6 Barron, 1976; Hachisuka, Umezu, & Ogata, 1997). Muscle atrophy induced by stroke is
7 associated with denervation and inactivity. Denervation of lower motor neuron induces muscle
8 fiber atrophy innervated by the motor neuron as development and function of skeletal muscle is
9 dependent upon a connection of central nervous system with lower motor neurons (Song, Ji, &
10 Ham, 1998). In skeletal muscles, type II fibers show greater atrophy than type I fibers following
11 denervation by stroke (Dattola, Girlanda, Vita, Santoro, Roberto, Toscano, Venuto, Baradello, &
12 Messina, 1993; Hachisuka, Umezu, & Ogata, 1997; Slager, Hsu, & Jordan, 1985).

13 Another potential factor contributing to muscle changes following stroke is undernutrition.
14 Anorexia, dysphagia, and functional disability of upper arm can reduce nutritional intake
15 (Axelsson, Notberg, & Asplund, 1984; Dennis, 2000; Unosson, Ek, Bjurulf, von Schenck, &
16 Larsson, 1994). Clinical studies have shown that 16% - 26% of acute stroke patients (Axelsson et
17 al., 1988; Dennis, 2000; Davalos, Ricart, Gonzalez-Huix, Solder, Marrugat, Molins, Suner, &
18 Genisl, 1996) have undernutrition. Undernutrition results in a loss of lean muscle mass (Shizgal,

1 1990; Nam, 1964; Essen, Fohlin, Thoren, & Saltin, 1981) and progressive muscle wasting
2 (Brozek, 1990). In the protein- deprived rats, all the fiber types were smaller compared to the
3 age-matched controls, and type II fibers atrophied while type I fibers simply failed to grow
4 (Oldfors & Sourander, 1985).

5 In the biopsied anterior tibial muscles of hemiplegic patients studied 1 to 7 months post stroke,
6 Scelsi et al. (1984) reported progressive decreases in the fiber diameter and changes in fiber type
7 distribution with predominant type II fiber atrophy and type I fiber increase. Biopsies from the
8 asymptomatic legs were either normal or showed age-related muscle alterations (Scelsi et al.,
9 1984). Slager et al. (1984) also found type II fiber atrophy in the biopsied anterior tibial muscle
10 on the hemiplegic side of stroke patients studied from 9 months to 25 years following the stroke.
11 However, they also found some type I fiber atrophy which was not as great as that noted in type
12 II fibers (Slager et al., 1984). Dattola et al. (1993) using lateral gastrocnemius muscle biopsies
13 from the affected side of patients with hemiparesis 2 months to 2 years after stroke demonstrated
14 that the percentage of type II fibers was reduced with decreased mean fiber diameter. Similarly,
15 bilateral vastus lateralis muscle biopsies from 21 hemiplegic persons obtained on average of 21.8
16 months following stroke revealed type II fiber atrophy with type I hypertrophy and no muscle
17 fiber atrophy or hypertrophy on the non-involved side (Hachisuka et al., 1997). In a rat model,
18 Haddad, Arnold, Zeng, and Baldwin (1997) found that selective denervation transformed the

1 plantaris, fast twitch (type II) muscle to a slow twitch (type I) muscle.

2 There have been few studies examining the effects of acute stroke on fiber cross-sectional
3 area and myofibrillar protein content. An, Lee, Im, Choi, and Choe (2000) were the first to report
4 on muscle mass of rat hind limb muscles following acute stroke. They found that muscle weight
5 of both affected and unaffected soleus, plantaris and gastrocnemius muscles were decreased at
6 seven days following stroke. However, they did not examine specific muscle characteristics such
7 as type I and II fiber cross-sectional area and myofibrillar protein content of three muscles. In
8 addition, it is not known whether the alterations in muscle weights result from undernutrition
9 associated with stroke or the lack of neuronal stimulation. Thus the current study was focused on
10 exploring muscle mass, myofibrillar protein content and type I and II fiber cross-sectional area in
11 rats following acute stroke and determining whether similar changes can be induced by
12 undernutrition alone.

13 **Methods**

14 Experimental Design: An experimental control group design was used to quantify skeletal
15 muscle atrophy (Figure 1). General anesthesia, sham operation, operation of stroke induction and
16 pair feeding to match intake of stroke rats was approved by the Animal Care Committee. Rats
17 were assigned randomly to either a control (C), undernourished (UndN), or stroke (S) group.
18 The control rats had a sham occlusion, the UndN rats were pair-fed to match intake of the S rats

1 and the S rats underwent right middle cerebral artery occlusion. Bilateral soleus, plantaris and
2 gastrocnemius muscles were dissected and brain was sectioned on the seventh day of the
3 experiment.

4 Sample: The study used 240-270g male Sprague-Dawley rats (Daehan Experimental Co.,
5 Korea). Ten rats were assigned to each group. Animals were housed in a 12-hour light, 12-hour
6 dark environment. Water and pellets (Samyang Co., Korea) were provided ad libitum for both
7 the C and the S rats. Dietary intake of the UndN group was determined based on daily dietary
8 intake of the S rats. Three rats in the C group died during the experimental period following the
9 sham operation, and three rats in the S group either died during the experimental period
10 following induction of stroke or were eliminated because stroke was not induced as determined
11 on autopsy.

12 Induction of Stroke: Stroke was induced by focal cerebral ischemic model (Nagasawa &
13 Kogure, 1989) (Figure 2). General anesthesia was introduced by gas mixed 70% N₂O gas and
14 28.5% O₂ with 1.5% enflurane. The right common carotid artery was exposed and internal
15 carotid artery was separated at the junction of right internal carotid artery and right external
16 carotid artery. A probe made from 1.8 cm long 4-0 nylon suture silk coated with silicon
17 (Xantropen, Bayer Dental) was inserted through a dissected portion of the upper part of internal
18 carotid artery to occlude the blood flow to right middle cerebral artery for two hours.

1 Following occlusion, the probe was removed to reperfuse with the right middle cerebral artery
2 and the site sutured. Throughout the procedure a rectal thermometer was used to monitor body
3 temperature. Temperature was maintained at $37^{\circ}\pm 0.7^{\circ}\text{C}$ by using warm pads.

4 Identification of Cerebral Infarction: At autopsy the brain was sectioned to identify the area of
5 cerebral infarction. Sectioned brain tissues were cut into seven slices by 2mm thickness, and
6 slices were dipped into 2% triphenyltetra-zolium chloride (TTC) and stained at room temperature
7 (Benderson, Pitts, Nishimura, Davis, & Bartkowski, 1986). Normal tissue stained with red while
8 infarcted area stained white.

9 Sham Occlusion: The right common carotid artery was exposed following general anesthesia
10 similar to stroke induction. Internal carotid artery was separated at the junction of right internal
11 carotid artery and right external carotid artery but was not occluded. Body temperature was
12 maintained similar to that described above.

13 Intake of Water and Diet: Rats were not fed immediately postoperatively to prevent aspiration.
14 Intake of diet was started one day following stroke induction. Thereafter, both the C and the S
15 groups were allowed to have water and pellet ad libitum. The UndN group was pair-fed to match
16 intake of the S rats and had water ad libitum.

17 Tissue Preparation: Rats were anesthetized with sodium pentobarbital (50mg/kg
18 intraperitoneal, supplemented as required) at seven days following stroke. Soleus, plantaris and

1 gastrocnemius muscles were excised bilaterally, the wet mass of individual muscles was
2 obtained, and then the tissue was rinsed with normal saline.

3 Measures: *Muscle weight*. The weight of dissected individual muscles was measured using a
4 microbalance (Mettler PE 160, USA) after trimming fat tissue and connective tissue.

5 *Cross-Sectional Area of Type I, II Fibers*. From each muscle, a portion was cut transversely
6 from the midsection, mounted in wooden pieces and quick frozen by immersion in isopentane
7 cooled with liquid nitrogen. Transverse sections 10 μ m thick were sectioned in a cryostat at –
8 20°C, adhered to cover glasses, thawed, and air-dried at room temperature for 30 minutes.

9 Myosin ATPase reactions were performed on serial sections, and fibers were classified as type I
10 (slow-twitch) or type II (fast-twitch) based on this reaction (Lee, Son, Jung, Cho, & Jin, 1990).

11 Pre-incubation for five minutes was carried out in a medium of both pH 4.3 acetate buffer and
12 pH 4.6 acetate buffer. The medium contained 200mM acetate and pH was adjusted by 2M HCl.

13 These sections were then rinsed with normal saline. Incubation was carried out in the medium

14 that contained 1.1M sodium barbiturate 20ml, 180mM CaCl₂ 10ml and 152mg ATP for 30

15 minutes at 37°C. The pH was adjusted to 9.4. Sections were rinsed with normal saline following

16 the incubation, then rinsed with 1% CaCl₂ three times every two minutes. Sections were reacted

17 with 2% CoCl₂ for three minutes, then rinsed with normal saline five times. Sections were

18 reacted with 2% ammonium sulfate for two minutes, then rinsed with normal saline five times.

1 Following histochemical staining and incubations, sections were dehydrated through a series of
2 ethanol concentrations from 70% to 100%, cleared in xylene and mounted. Type I fibers were
3 identified as those staining dark while type II fibers were identified as those staining light in
4 ATPase reactions after pre-incubation at pH 4.3 and at pH 4.6 (Brooke & Kaiser, 1970). Fiber
5 cross-sectional area was calculated from tracings of 50-100 muscle fibers at 100x magnification
6 by microscopic image analyzer (Leity, ASM 68k, Netzler) (Lee, Son, Jung, Cho, & Jin, 1990).

7 *Myofibrillar Protein Content.* Frozen soleus, plantaris and gastrocnemius muscles were first
8 homogenized in buffer containing 1ml borate-KCl buffer solution and 10 μ l of 1mM DTT (DL-
9 dithiothreitol) by vortex mixer. The homogenate was centrifuged at 4°C and 3200 rpm for
10 15 minutes. Supernatant was removed and membrane bound protein were removed by infusing
11 the second buffer (Tris-Cl 1.576g + 0.1M KCl 200 ml) 1 ml and 10 μ l of 1 mM DTT into the
12 remaining pellet. Supernatant was removed and the third buffer (MgCl₂ 0.08132g +20mM
13 EGTA 1.6ml + Tris-maleate 0.4744g +0.1 KCl 198.4ml) 1ml was infused and mixed and
14 centrifuged for 15 minutes. Supernatant was removed and the third buffer 1ml was infused and
15 centrifuged for 15 minutes. Supernatant was removed again. I N NaOH 0.5ml was infused into
16 the remaining pellet and mixed to use for quantification of the protein. 1ml Bio-Rad (Bio-Rad
17 Laboratories, USA) mixed with a diluted solution 100 times from 10 μ l sample was analyzed by
18 Bradford assay method (Bollag, Rozycki, & Edelstein, 1996) using a spectrophotometer

1 (UV1601, SHIMADZU, Japan). Bovine serum albumin was used as a standard solution, and
2 absorbance (A595) was indicated on the spectrophotometer. Myofibrillar protein content (mg/g)
3 was calculated by the following equation using a value (ug/ml) indicated on the
4 spectrophotometer:

$$5 \text{ Myofibrillar protein content (mg/g)} = \frac{\text{absorbance} \times \text{diluted times} \times \text{muscle weight(1g)}}{\text{Individual muscle weight}} .$$

6 Data Analysis: Data are presented as means and standard deviations of the mean ($M \pm SD$) for
7 all variables except fiber type distribution. Between – and within – group comparisons were
8 determined by Kruskal Wallis test followed by Mann-Whitney U test a post hoc test. Statistical
9 significance was accepted at the level of $P < 0.05$.

10 Results

11 Changes of Body Weight: At baseline there were no significant differences in body weights
12 among three groups (Table 1). Seven days following acute stroke or sham procedure, body
13 weight of the S group was decreased to 78% compared to the C group ($p = 0.03$); that of the
14 UndN group was decreased to 82% compared to the C group ($p = 0.02$). Body weight of the C
15 group increased to 11.0% over baseline; that of the UndN group decreased to 88% of baseline (p
16 $= 0.04$); that of the S group decreased to 83.7% of baseline ($p = 0.03$).

17 Total Amount of Diet Intake: When compared to the C group, the S and the UndN group
18 consumed significantly less food ($p = 0.001$, $p = 0.001$) (Table 2).

1 Hindlimb muscle weight: Muscle weights of both affected and unaffected soleus of the S
2 group decreased to 84%, 88% each of the C group ($p = 0.02$, $p = 0.02$) (Table 3). Muscle weights
3 of both affected and unaffected gastrocnemius of the S group decreased to 78%, 81% each of the
4 C group ($p = 0.03$, $p = 0.02$). Muscle weights of both affected and unaffected plantaris of the S
5 group tended to be smaller, although not statistically significant, than that of the C group. Muscle
6 weights of both affected and unaffected three muscles of the UndN group tended to decrease
7 compared to the C group and were not statistically different from the S group.

8 Fiber type distribution and cross-sectional area of soleus muscle: The effects of stroke on
9 fiber type distribution and cross-sectional area of soleus muscle are shown in Table 4. Type I
10 fiber cross-sectional area of both affected and unaffected soleus muscles of the S group
11 decreased to 72% each of the C group ($p = 0.03$). Type II fiber cross-sectional area of both
12 affected and unaffected soleus muscles of the S group tended to decrease when compared to the
13 C group. There was no difference in fiber type distribution of both affected and unaffected soleus
14 muscles between the S and the C group. Type I and II fiber cross-sectional area of both affected
15 and unaffected soleus muscles of the UndN group tended to decrease compared to the C group
16 and were not statistically different from the S group.

17 Fiber type distribution and cross-sectional area of plantaris muscle: As shown in Table 5, type
18 I and II fiber cross-sectional area of both affected and unaffected plantaris muscles of the S

1 group tended to be smaller than the C group. When compared to the C group, type I and II fiber
2 distribution of both affected and unaffected plantaris muscles of the S group were not
3 significantly different. Type I and II fiber cross-sectional area of both affected and unaffected
4 plantaris muscles of the UndN group tended to decrease compared to the C group and were not
5 statistically different from the S group.

6 Fiber type distribution and cross-sectional area of gastrocnemius muscle: The effects of stroke
7 on fiber type distribution and cross-sectional area of gastrocnemius muscle are presented in
8 Table 6. Comparison of the C group versus the S group revealed no significant difference in type
9 I fiber cross-sectional area of both affected and unaffected gastrocnemius muscles. Type II fiber
10 cross-sectional area of both affected and unaffected gastrocnemius muscles of the S group tended
11 to be smaller than the C group. No significant difference in type I and II fiber distribution of both
12 affected and unaffected gastrocnemius muscles of the S group was identified compared to the
13 C group. Type I and II fiber cross-sectional area of both affected and unaffected gastrocnemius
14 muscles of the UndN group tended to decrease compared to the C group and were not
15 statistically different from the S group.

16 Myofibrillar Protein Content of Hindlimb Muscles: As shown in Table 7, myofibrillar protein
17 content of affected soleus muscle of the S group significantly decreased to 67% of the C group
18 ($p = 0.03$), that of unaffected soleus muscle of the S group significantly decreased to 75% of the

1 C group ($p = 0.03$). Myofibrillar protein content of both affected and unaffected plantaris and
2 gastrocnemius muscle of the S group tended to be smaller than the C group. Myofibrillar protein
3 content of both affected and unaffected plantaris and gastrocnemius muscle of the UndN group
4 tended to decrease compared to the C group and was not statistically different from the S group.

5 **Discussion**

6 The present study examined whether muscle atrophy following acute stroke is induced by
7 undernutrition or denervation or both. The major finding of this study was that significant
8 decreases were found in both affected and unaffected soleus and gastrocnemius muscle weight,
9 type I fiber cross-sectional area and myofibrillar protein content of the stroke group as compared
10 to the control group.

11 We had hypothesized that muscle wasting in stroke rats would be due to denervation.
12 Several observations supported this hypothesis. Kondo, Nagara and Tateishi (1987) reported that
13 the total number of myelinated and large fibers of the lumbar ventral roots were significantly
14 decreased in autopsied patients with cerebrovascular diseases. Qiu, Wada, and Otomo (1991)
15 found that in autopsied post stroke patients, the cross-sectional area of cervical anterior horn
16 cells on the affected side was significantly decreased compared with that on the unaffected side
17 and the controls. However, findings from the present study that similar decreases in muscle
18 characteristics were noted in both affected and unaffected muscles suggests that denervation may

1 not be the only factor contributing to muscle changes at seven days post stroke. If the
2 experimental protocol had been designed to allow for later observations, e.g., 1 month post injury,
3 greater differences between the two sides may have been observed. Indeed based on clinical
4 studies, type II fiber atrophy was present in the biopsied anterior tibial muscles of hemiplegic
5 patients studied 1 to 7 months post stroke but not in biopsies on the affected side (Scelsi et al.,
6 1984). This muscle fiber atrophy has been validated by other clinical studies using muscle
7 biopsies 2 months to 25 years post stroke (Slager et al., 1984; Dattola et al., 1993; Hachisuka et
8 al., 1997). Thus additional factors need to be considered.

9 Because inadequate nutrition is known to influence muscle characteristics, the current study
10 utilized a pair-fed control group. As expected, this pair-fed group demonstrated reductions in
11 caloric intake and body weight similar to that observed in the stroke group. Muscle alterations
12 such as muscle mass, type I and II fiber cross-sectional area, myofibrillar protein content of the
13 undernourished group tended to be similar to those observed in the stroke group. However, there
14 were no statistical differences between the undernourished and the control group. The role of
15 undernutrition following stroke was suggested by the work of An and others (2000). They found
16 that the muscle weights of both affected and unaffected hindlimb muscles following acute stroke
17 were decreased compared to control rats and that diet intake of stroke rats was less than that of
18 control rats at seven days post injury. However, they did not study a pair-fed group similar to the

1 current study. Thus during the first week following cerebral vessel occlusion, undernutrition may
2 contribute to muscle loss in both the denervated and unaffected muscles.

3 Another contributing factor to muscle atrophy post stroke is inactivity. Space flight, various
4 models of inactivity including hindlimb suspension and whole body suspension have been used
5 to induce muscle atrophy. Caiozzo, Baker, Herrick, Tao, and Baldwin (1994) found that a small
6 increase in the percentage of the type II fibers and a greater atrophy of the type I fibers following
7 a six-day space flight. Isfort and colleagues demonstrated that seven days of hindlimb suspension
8 resulted in 20% reduction in soleus muscle mass (Isfort, Wang, Greis, Sun, Keough, Farrar,
9 Bodine, & Anderson, 2002). Similarly, Alley and Thompson showed a 37% reduction in soleus
10 muscle mass after four to seven days of hindlimb suspension (Alley & Thompson, 1997) and we
11 observed an 18% decrease following seven days of hindlimb suspension (Choe, Park, & Koh,
12 1994). In a follow-up study, soleus, plantaris and gastrocnemius muscle weights were decreased
13 34%, 8%, and 11% respectively following seven days of hindlimb suspension (Choe & Shin,
14 1999). Sandmann, Shoeman, and Thompson (1998) demonstrated a number of changes following
15 seven days of hindlimb suspension including 18% decrease in the ratio of gastrocnemius weight
16 to body weight, decreased muscle fiber diameter and decreased peak active force of type I fibers
17 of gastrocnemius muscle.

18 The present study found that soleus muscle weight of stroke rats decreased significantly while

1 plantaris muscle weight tended to decrease at seven days post stroke. This is consistent with the
2 observations of Musacchia, Steffen, and Deavers (1983) who found that hindlimb skeletal
3 muscle unloading by whole body suspension is associated with a pronounced atrophy of the slow
4 twitch soleus while the fast twitch plantaris is relatively insensitive to this form of disuse.
5 Antigravity affects slow-twitch type I muscles more than primarily fast-twitch type II muscles
6 (Fitts, Metzger, Riley, & Unsworth, 1986; Tischler, Henriksen, Munoz, Sump, Woodman, &
7 Kirby, 1993). Thus muscle changes observed in the current study are consistent with those
8 observed in non-denervated suspended muscle suggesting that inactivity is an important factor.

9 In the present study, we did not objectively measure physical activity in any of the 3 groups.
10 However, daily observations of the animals indicated that those in the stroke group demonstrated
11 flexion of the upper limb of the affected side. This flexion created difficulty in walking and
12 moving about the cage.

13 The reduction of myofibrillar protein content following acute stroke in this study is in
14 agreement with our previous results which found that inactivity (seven days of hindlimb
15 suspension) reduced myofibrillar protein content of plantaris and soleus muscle 51% and 60%
16 respectively (Choe, Ji & Kim, 1995). Seven days of hindlimb suspension also decreased
17 myofibrillar protein content of soleus, plantaris and gastrocnemius muscles 48%, 41%, and 37%
18 respectively (Choe et al., 1999). Myofibrillar degradation is an early event in the atrophy of

1 hindlimb muscles in response to inactivity (Baldwin, Herrick, Ilyina-Kakueva, & Oganov, 1990).
2 Muscle protein wasting by inactivity is caused by reduction of protein synthesis and increased
3 protein breakdown (Thomason & Booth, 1990). Thomason, Biggs, and Booth (1989) found that
4 myofibrillar protein synthesis rate was decreased 59% in the soleus muscle after seven days of
5 hindlimb suspension suggesting that this may be responsible for myofibrillar protein reductions.

6 In the current study, type I fiber cross-sectional area of both affected and unaffected soleus
7 muscle of stroke rats significantly decreased while type II fiber cross-sectional area tended to
8 decrease. Our finding is not consistent with the findings of previous human studies in which type
9 II atrophy of affected lower limb muscles were found in hemiplegic patients beginning at one
10 month post stroke with no muscle fiber atrophy on the unaffected side (Scelsi et al., 1984;
11 Hachisuka et al., 1997). However, the present study results are in agreement with previous
12 animal studies: type I fiber cross-sectional area of both soleus and plantaris muscle are decreased
13 following seven days of hindlimb suspension (Choe et al., 1994, 1995); the decrease in cross-
14 sectional area of type I (slow twitch) fibers in the unweighted soleus is rapid, occurring during
15 the first two weeks of unweighting (Templeton, Sweeney, Timson, Padalino, & Dudenhofer,
16 1988); and type I fibers showed greater inactivity-induced atrophy than type II fibers (Thompson
17 & Brown, 1999;Thompson, 1999). Our results suggest that decreased type I fiber cross-sectional
18 area following acute stroke may be associated with inactivity.

1 Collectively, muscle atrophy noted seven days following acute stroke may be due to
2 undernutrition and inactivity rather than the denervation per se. However, with time, greater
3 changes in the affected as compared to unaffected side muscles are likely to emerge. Such
4 changes may reflect the greater impact of neuronal stimulation loss.

5 In summary, we identified that the decrease in muscle mass, type I fiber cross-sectional area
6 and myofibrillar protein content at seven days of stroke is more apparent in soleus muscle than
7 both plantaris and gastrocnemius muscle. Muscle atrophy following acute stroke may be
8 attributed to undernutrition and inactivity in addition to loss of neuronal stimulation. These
9 results support the need for continued attention to nutrition as well as activity during the acute
10 phase following stroke.

11 Recognition of the risk of undernutrition and inactivity during the acute stage of stroke, for
12 acute stroke patients, must rest with nurses. Nurses should play a central role in prevention of
13 muscle atrophy induced by undernutrition and inactivity following acute stroke. It remains to be
14 determined whether greater efforts spent on maintaining adequate nutritional intake and
15 providing muscle stimulation during this acute stage will reduce long-term muscle disability
16 associated with stroke.

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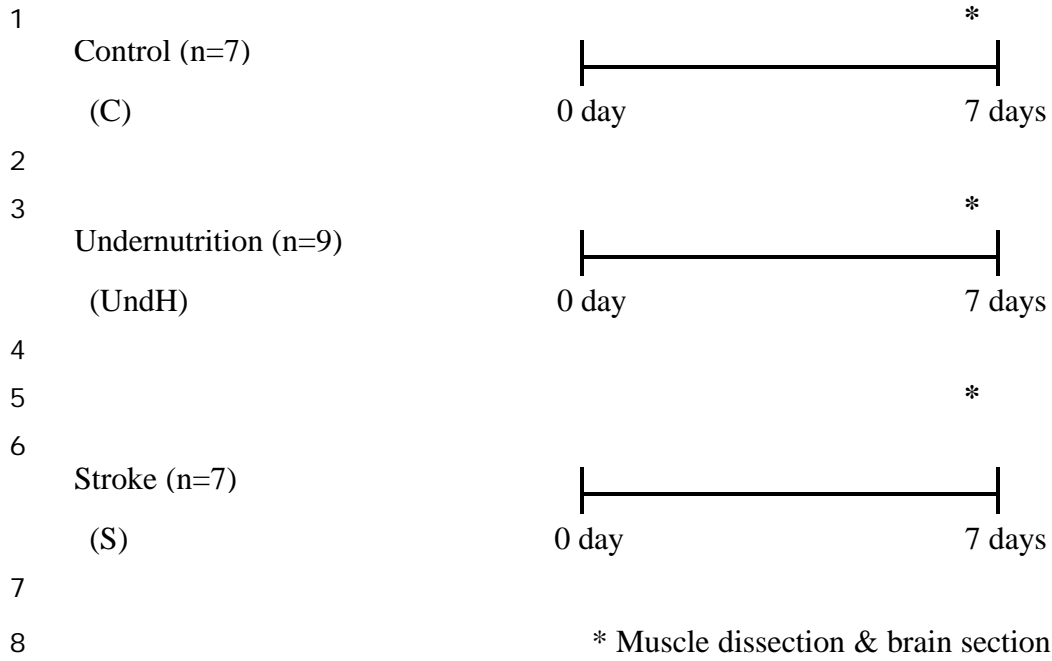
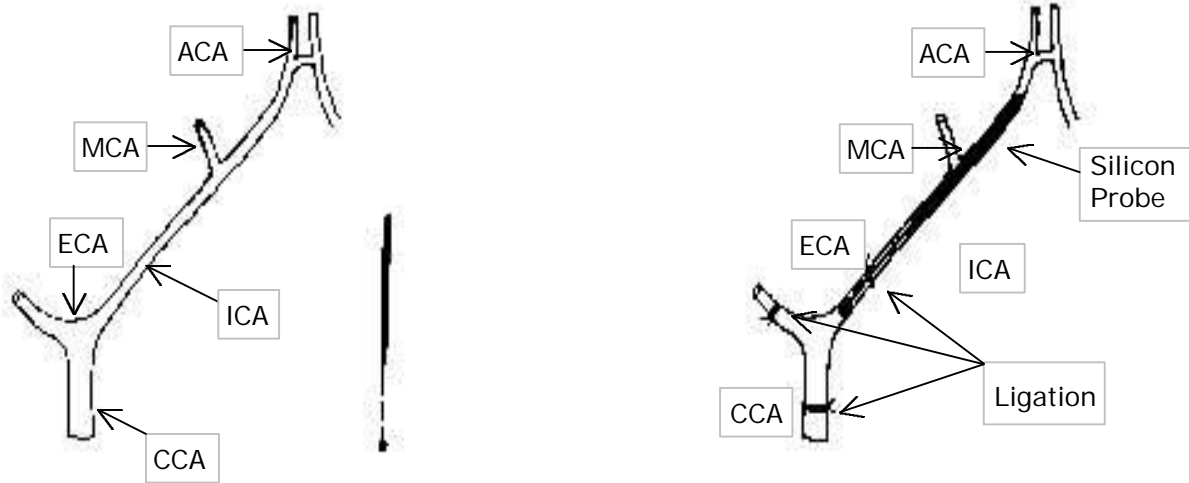


Figure 1. Experimental Design



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Figure 2. Focal cerebral ischemia model. This figure shows that the occlusion using silicon probe obstructs the blood flow to MCA (middle cerebral artery) in focal cerebral ischemia model, which is developed by Nagasawa and Kogure (1989). This figure was cited from Park (2002).

1 Table 1

2 Pre and post body weight of control(C), undernourished (UndN) and stroke (S) rats

	prewt.(g)	postwt.(g)	post/pre(%)
C (n=7)	241.3±14.4	266.0±10.8	110.2
UndN (n=9)	246.7±21.9	217.9±13.3¶	88.0
S (n=7)	248.0±5.4	207.5±5.2*+	83.7

3 n; number of animals.

4 prewt.: body weight at the start of experiment. postwt.: body weight before muscle dissection.

5 * Significantly different between C & S group (p<.05)

6 + Significantly different between prewt. & postwt.(p<.05)

7 ¶ Significantly different between C & UndN group (p<.05)

8

1 Table 2

2 Total amount of diet intake and weight gain during the experimental period

	Total diet intake (g)	weight gain(g)
C (n=7)	124.8±19.5	+24.7
UndN (n=9)	46.2±0.0¶	-28.8¶
S (n=7)	46.5±30.5*	-40.5*

3 n; number of animals.

4 C: Control, sham operation, S: Stroke, UndN: Undernourished

5 * Significantly different between C & S group (p<.05)

6 ¶Significantly different between C & UndN group(p<.05)

7

1 Table 3

2 Muscle weight of hindlimb muscles in control(C), undernourished (UndN) and stroke (S) rats

Group	Left (affected side)			Right (unaffected side)		
	Mean±SD (mg)			Mean±SD (mg)		
	Soleus	Plantaris	Gastrocnemius	Soleus	Plantaris	Gastrocnemius
C (n=7)	106.0± 19.3	229.3±31.3	1270.9±73.1	106.3± 22.4	218.6±38.6	1241.6±167.5
UndN (n=9)	101.2± 12.5	207.3±23.1	1183.0±142.5	104.1± 12.5	224.2±43.3	1215.4±152.2
S (n=7)	89.9± 16.9*	204.1±50.9	989.7±270.9*	94.4± 19.1*	200.9±61.7	1001.5±278.6*

3 n: number of animals

4 * Significantly different between C & S group (P<.05)

5

1 Table 4

2 Fiber type distribution and cross-sectional area of soleus muscle in control(C), undernourished
3 (UndN), and stroke (S) rats

Group	Left soleus (affected side)				Right soleus (unaffected side)			
	Fiber CSA (μm^2)		Fiber type distribution (%)		Fiber CSA (μm^2)		Fiber type distribution (%)	
	mean \pm SD				mean \pm SD			
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
C (n=7)	2793.0 \pm 554.9	2362.0 \pm 415.3	82.7	17.3	2846.0 \pm 406.3	2383.9 \pm 441.9	84.0	16.0
UndN (n=9)	1980.5 \pm 188.3	1892.5 \pm 207.6	90.0	10.0	1944.9 \pm 167.3	1946.2 \pm 121.5	90.3	9.7
S (n=7)	2000.8 \pm 83.9*	1818.1 \pm 217.5	91.2	9.8	2053.0 \pm 100.1*	1853.9 \pm 152.9	90.2	9.8

4 n: number of animals CSA: cross-sectional area

5 * Significantly different between C & S group (P<.05)

6

1 Table 5
 2 Fiber type distribution and cross-sectional area of plantaris muscle in
 3 Control (C), undernourished (UndN) and stroke (S) rats

Group	Left plantaris (affected side)				Right plantaris (unaffected side)			
	Fiber CSA (μm^2)		Fiber type distribution (%)		Fiber CSA (μm^2)		Fiber type distribution (%)	
	Mean \pm SD				Mean \pm SD			
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
C (n=7)	2105.0 \pm 187.7	1839.6 \pm 79.2	10.5	89.5	2115.0 \pm 156.6	1846.2 \pm 70.5	10.2	89.8
UndN (n=9)	1852.1 \pm 124.4	1690.9 \pm 180.6	11.0	89.0	1911.7 \pm 94.6	1692.5 \pm 176.1	10.3	88.7
S (n=7)	1829.1 \pm 158.9	1566.1 \pm 142.0	13.8	86.2	1829.6 \pm 145.3	1538.2 \pm 208.2	12.0	88.0

4 n: number of animals. CSA: cross-sectional area

5

1 Table 6

2 Fiber type distribution and cross-sectional area of gastrocnemius muscle in control(C),
3 undernourished (UndN) and stroke (S) rats

Group	Left gastrocnemius (affected side)				Right gastrocnemius (unaffected side)			
	Fiber CSA (µm ²)		Fiber type distribution (%)		Fiber CSA (µm ²)		Fiber type distribution (%)	
	Mean±SD				Mean±SD			
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
C (n=7)	1594.2± 173.9	1530.7±74.1	11.7	88.3	1624.3± 234.5	1533.7±14.1	10.0	90.0
UndN (n=9)	1733.1± 213.8	1458.2±98.3	5.1	94.9	1687.1± 157.8	1454.7±182.0	7.4	92.6
S (n=7)	1666.7± 202.9	1423.9±96.5	16.6	83.4	1607.7± 177.2	1523.8±95.6	10.4	89.6

4 n: number of animals. CSA: cross-sectional area

5

1 Table 7

2 Myofibrillar protein content of hindlimb muscles in control(C), undernourished (UndN) and
 3 stroke (S) rats

Group	Left(affected side)			Right(unaffected side)		
	Mean±SD(mg/g)					
	Soleus	Plantaris	Gastrocnemius	Soleus	Plantaris	Gastrocnemius
C (n=7)	78.1± 10.1	83.8±13.0	91.7±3.8	77.9±8.0	84.6±8.0	88.0±6.8
UndN (n=9)	59.0± 11.2	70.7±12.1	84.4±6.1	63.2±8.0	79.9±7.8	84.3±5.9
S (n=7)	52.9± 8.6*	62.9±8.6	85.5±6.2	58.5±7.3*	79.8±7.6	83.6±9.8

4 n: number of animals

5 * Significantly different between C & S group (P<.05)