

Nursing Research Checklist for style

General

Supply permission from publisher to reproduce published materials: The citation of the figure from unpublished master's thesis have addressed in the reference list of the manuscript according to the "unpublished master's thesis, university outside the United States" on page 262 of APA Manual (5th ed). See revised manuscript page 27

Title page

Shorten title to 12 words or less: We have done this.

References

After the 6 author's name and initial, use et al. to indicate the remaining authors of the article: We have done this. See revised manuscript page 26 and 27.

In-text citation; For 6 or more authors, use only the first author's name with et al; We have done this. See page revised manuscript 5, 6, 7, 10, 22 and 23.

Add missing author to Qiu(1991): We have added author to Qiu. See page 20 of revised manuscript.

Correct year of publication for Korean Stat Office: We have done this. See page 27 of revised manuscript.

Figures

Attach to each manuscript good quality copies of all figures: We have attached good quality copies of all figures.

Reviewer 1

1. **Multiple purpose statements:** In accordance with the reviewer's comment, we have used the purpose statement from the abstract, in the introduction and discussion.
2. **Hypothesis:** In response to the reviewer's request we have added three hypotheses. These are: 1) denervation following acute stroke will decrease muscle mass, type I and II fiber cross-sectional area and myofibrillar protein content of affected hindlimb muscles of rat; and 2) undernutrition will decrease muscle mass, type I and II fiber cross-sectional area and myofibrillar protein content of rat hindlimb affected and unaffected muscles; and 3) there will be no differences in muscle characteristics between affected and unaffected hindlimb muscles at 7 days post ischemic stroke. (see revised manuscript page 8).
3. **Physiological rationale for the study:** We have added an additional statement indicating that acute stroke (in the middle cerebral artery) results in loss of upper motor function and subsequently lower motor neuron loss, muscle inactivity, and eventually muscle atrophy (see page 5 of revised manuscript).
4. **More complete explanation of the design: justification for having included the underfed but non-sham operated group:** We have added a sentence to indicate that an underfed group was used to control for reduced dietary intake which occurs subsequent to stroke induction (see revised manuscript page 9). We acknowledge that a perfect design would have included a non-sham operated group. However, we believed that there would be no difference between sham operated and non-sham operated groups on the outcome measures for this study. This could be viewed as a limitation and a statement regarding this has been added to the discussion (see revised manuscript page 23).
5. **Probe:** In response to the reviewer's concern regarding the probe. We have added and revised sentences to indicate that a silicone rubber cylinder attached to nylon suture silk was inserted through a dissected portion of the upper part of internal carotid artery to occlude the blood flow to the right

middle cerebral artery (RMCA) for two hours. The cylinder was made of 4-0 nylon suture silk 16mm long coated with silicone(Xantopren, Bayer Dental) mixed with a hardener(Elastomer Activator) to thicken the distal 5mm to 0.25-0.30mm(Nagasawa & Kogure,1989) (see page 10 of revised manuscript). The silk thread was coated with silicon making it stiff and inflexible. Thus, when positioned in the artery after dissection, blood flow was completely occluded. This was verified by visual observation during the occlusion as well as autopsy results.

6. **Data analysis:** In response to the reviewer's question, we originally used Kruskal Wallis test for within group comparisons (3 group comparisons) and Mann-Whitney U test for between group comparisons (i.e., 2 group comparisons; control vs stroke, control vs undernourished, stroke vs undernourished). Based on the reviewer's comments, we reanalyzed our data using ANOVA and Duncan's test. The manuscript has been revised to reflect that an overall effect of the treatment was first tested and when group differences were significant Duncans was used to examine what group differences existed.
7. **An explanation regarding comparison between affected and unaffected legs in the design:** In accordance with the reviewer's comment we have added an additional hypothesis in the purpose indicating that there will be no differences in muscle characteristics between affected and unaffected hindlimb muscles at 7 days post ischemic stroke (see page 8 of revised manuscript).
8. **Discussion; restate each of the hypothesis and indicate clearly what was found in relation to if the hypothesis was or was not supported:** Based on this recommendation we have revised the manuscript accordingly (see the last line of page 18 and page 19 of revised manuscript).
9. **p20,17 and 18 this sentence does not make sense. The reviewer is referring to the sentence 'Our results suggest that decreased type I fiber cross-sectional area following acute stroke may be associated with inactivity.':** We revised the discussion based on the findings from the reanalysis.
10. **p21,12 and 13, what does it mean that nurses" should have a central role":** Our point was that prevention of muscle atrophy following acute stroke requires a collaborative multidisciplinary approach, and that nurses have an important role among healthcare professionals to prevent from complications of acute stroke.
11. **p21, 12, and 11, Inactivity was not a variable in this study and no measure of activity was reported :** We used the term 'inactivity' to reflect that it is often a natural consequence of the stroke or ischemia in the middle cerebral artery. The loss of the upper motor neurons due to ischemia results in paralysis/paresis of extremity muscles. We included the term in this statement to reflect additional factors that may have contributed to the observed outcomes. The reviewer is correct that the current study contained no measures of inactivity as reflected in the statement on page 21 and 22 (revised manuscript). However, daily observations of the animals indicated that those in the stroke group demonstrated flexion of the upper limb of the affected side. This flexion created difficulty in walking and moving about the cage providing at least anecdotal evidence that inactivity may have contributed to the observed findings.
12. **Definition of muscle atrophy:** In response to the reviewer's suggestion, we added a definition on page 5 (revised manuscript). Muscle atrophy is defined as a decrease in muscle mass, cross-sectional area, and myofibrillar protein content.
13. **Figure 1 is superfluous:** In response to reviewer's comment this figure has been deleted.

14. **Figure 2 abbreviation:** In response to reviewer's comments we have added abbreviations indicating that CCA; common carotid artery, ECA; external carotid artery, ICA; internal carotid artery, ACA; anterior cerebral artery, MCA; middle cerebral artery. (see figure2, revised manuscript).

Reviewer 2

1. Abstract:

- a. **Delete p values and give text, significantly increased or decreased compared to controls:**
Because we reanalyzed our data based on the reviewers' comment, we were able to reduce the number of p values in the abstract.

b. **"In the last line of results, insert control group values..."**

We reanalyzed our data and revised the results of abstract based on the findings from the reanalysis.

2. **Introduction:** In accordance with the reviewer's comment we have deleted "for example" in line 1 and "from stroke" in line 8, and have added "on skeletal muscle" in line 12 (see revised manuscript page 5), and have changed "related to disuse and lack or limited neurologic stimulation" into "disuse or reduced_neurologic stimulation" on page 6 of revised manuscript.

3. Page 6:

- a. **"Take out the last sentence"** We have made the suggested editorial changes on page 6 of revised manuscript.

b. **"Then have a new paragraph..."** In response to reviewer's comments, we have made a new paragraph: In humans muscle fiber size and cross-sectional area of the affected side decrease resulting in muscle atrophy (Hachisuka, Umez & Ogata, 1997). Denervation of lower motor neuron induces muscle fiber atrophy as development and function of skeletal muscle are dependent upon a connection between the central nervous system and lower motor neurons (Song, Ji, & Ham, 1998). In skeletal muscles, type II fibers show greater atrophy than type I fibers following denervation by stroke (Dattola et al., 1993; Hachisuka et al., 1997) (see page 6 of revised manuscript).

c. **Rewording:** In response to reviewer's editorial comments, we have reworded that another potential factor contributing to muscle "change" into muscle "atrophy" and "have undernourished" into "are undernourished" in the last line of page 6 and page 7 of revised manuscript. Reviewer suggested muscle "loss", however, we contend that atrophy would be more appropriate than loss.

4. Page7:

a. **"Much of this materials should be summarized..."** In response to reviewer's comments we have summarized from line 5 to 13 indicating that type II fiber atrophy on hemiplegic (i.e., affected) side was found in biopsied anterior tibial muscles of hemiplegic patients studied 1 to 7 months (Scelsi, Lotta, Lommi, & Marchetti, 1984), 9months to 25 years (Slager, Hsu, & Jordan, 1985) following the stroke. Dattola et al. (1993) observed that the percentage of type II fibers was reduced in the affected lateral gastrocnemius muscle of hemiparesis patients at 2months to 2 years after stroke. Hachisuka et al.(1997) reported type II fiber atrophy with type I hypertrophy of vastus lateralis muscle on the hemiplegic side of 21 hemiplegic persons on average of 21.8 months following stroke. Two studies examined muscle changes on the unaffected side. Scelsi et al. (1984) found either normal or age-related muscle alterations in the unaffected side. Hachisuka et al. (1997) reported no muscle fiber atrophy or hypertrophy on the un-affected side. (see page 7 of revised manuscript)

b. **"Line 13 (line 17) (rat model) should begin a new paragraph..."** We acknowledge reviewer's suggestion, however, we did not begin a new paragraph because one sentence would not be enough to make a new paragraph (see the last line of page 7 and 8 of revised manuscript).

c. **"I do not think you need Figure 1..."** We have deleted this figure.

- d. **“Only the right part of Figure 2 is needed.”** We have deleted the left part of figure 2 (see figure 2 revised manuscript).
5. **Page 13, Data analysis:**
- a. **State what the Kruskal Wallis test is and why you are not using a parametric ANOVA ?”** In response to the reviewer’s question, we originally used Kruskal Wallis test for within group comparisons (3 group comparisons) and Mann-Whitney U test for between group comparisons (i.e., 2 group comparisons; control vs stroke, control vs undernourished, stroke vs undernourished). In response to reviewer’s comments, we have analyzed our data by ANOVA and Duncan’s procedure as a post-hoc test for group differences.
“Since you stated $p < 0.05$ was set as the level of significance, do not need all these p values...” We have done this based on the findings of reanalysis. In the case of paired t test, comparison between preweight and postweights, we have written p values (see page 14 of revised manuscript).
- b. **Results, line 3: “these percentiles do not match...”** We have indicated in the manuscript that the percentiles in the text are comparisons to the control group on day 7. The percentiles on Table 1 are comparisons of each group to its own baseline have been deleted (see table 1 revised manuscript).
- c. **Page 14 and Table 3 and Table 4: ‘For each muscle, is the UndN group different from control, or’** We reanalyzed our data and revised the manuscript based on the findings from the reanalysis.
6. **Page 15:**
- a. **“It looks like not differences between S and C, C and U, and S and U.”** We reanalyzed our data and revised it based on the findings from the reanalysis.
- b. **Were they (affected and unaffected) analyzed separately?”** We compared affected and unaffected hindlimb muscles by non-paired t test and found that there were no differences between them.
7. **Page 16 Discussion:**
- a. **Do be careful that in the discussion your findings are specific to what you did find (which muscles, which outcome) and not general.** In accordance with reviewer’s comments, the manuscript has been revised.
- b. **“Based on earlier studies(refs here) we hypothesized that----(which muscles were significant different from control??), suggesting that---“.** In response to reviewer’s comments we have changed our statement to indicate that “Based on earlier studies(Kondo, Nagara, & Tateishi, 1987; Qiu, Wada, Otomo & Tsukagoshi, 1991) we had hypothesized that muscle wasting would be due to denervation. However, we found that similar decreases in type I fiber cross-sectional area and myofibrillar protein content were present in both the affected and unaffected soleus muscle, and there were no differences in muscle characteristics between the affected and unaffected hindlimb muscles, suggesting” that ---. (see page 20 of revised manuscript)
- c. **There are lots of studies cited in the discussion that are not clearly tied to the findings of the present study. Needs to be tightened up.** In accordance with reviewer’s comments, we have revised the manuscript.

Reviewer 3

- Bar graphs:** In response to reviewer’s comments, we have made bar graphs of table 3 (muscle weights), table 4 (cross-sectional area of soleus muscle) and table 7 (myofibrillar protein content) to illustrate the difference. (see figure 2, 3 and 4 revised manuscript).
- Title change:** In response to reviewer’s suggestion we have changed a title: Effect of inactivity and undernutrition following acute ischemic stroke in a rat hindlimb muscle model.

3. **Table 1;** “ These changes would be nicely shown with line graph – did you try anacova -?”
In response to reviewer’s comments, we have analyzed our data by ANOVA and Duncan’s procedure for individual group differences.
4. **Results, discussion and abstract, refer to decreases in muscle weights and protein content, etc.- this is not correct as it does not appear that you measured these things at baseline. They are different from one another:** We would like to thank the reviewer for this very logical comment. The manuscript has been revised to clarify the wording.
Nonparametric test: In response to reviewer’s comments, we have analyzed our data by ANOVA and Duncan’s procedure as a post-hoc.

Reviewer 4

1. **Title change:** In response to reviewer’s suggestion we have changed a title: Effect of inactivity and undernutrition following acute ischemic stroke in a rat hindlimb muscle model.
2. **Objective:**
 - a. **Including clear hypothesis in the methods section or introduction.** In response to the reviewer’s suggestion we have added three hypotheses in the introduction. See response #2 to Reviewer #1.
 - b. **Clearly address hypothesis in their results and discussion section;** The manuscript has been revised. See responses to reviewers above.
3. **Introduction:**
 - a. **Review of the literature; needs to be tightened-up and condensed. “key” supporting studies can be neatly presented and their main findings summarized:** In response to reviewer’s suggestion, review of the literature was tightened- up and condensed (see responses above regarding specific changes to literature review).
 - b. **Reference:** In response to reviewer’s request we have eliminated some of the older references. Deleted references include Chokroverty et al.,1976; Axelsson et al., 1984; Nam, 1964; Essen, Fohlin, Thoren, & Saltin, 1981. In the case of - Brooke & Kaiser (1970), although dated the methods outlined in this paper continue to be used and thus we retained this reference.
4. **Methods :**
 - a. **Justify why this rodent model of stroke of chosen over other experimental models of stroke:** We have added statement in the methods indicating that a rat model of middle cerebral artery occlusion was used because it is reproducible and the most relevant to the human condition of ischemic stroke (Garcia, 1984; Longa ,Weinstein, Carlson, & Cummins, 1989; Nagasawa & Kogure,1989) on page 9 of revised manuscript.
 - b. **Group of (3) had n=9, versus n=7 in group 1 & 2- why different number of subjects:** We have indicated on page 9 and 10 (revised manuscript) that three rats in the C group died during the experimental period following the sham operation, and three rats in the S group either died during the experimental period following induction of stroke or were eliminated because stroke was not induced as determined on autopsy”.
 - c. **Was statistical power analysis done to justify the number of rats employed in this experiment?:**
A power analysis was not performed because we thought 10 rats/ group was adequate to detect differences using parametric methods. If differences were not observed in 10/group, then the clinical relevance would be questionable. Since completing the study we have conducted a power analysis as follows: Small effect size; $f=0.10$, $\alpha=0.05$, $u=2$, $n=10$: Power of F test=0.07,
Medium effect size; $f=0.25$, $\alpha=0.05$, $u=2$, $n=10$: Power of F test=0.20,
Large effect size; $f=0.40$, $\alpha=0.05$, $u=2$, $n=10$: Power of F test=0.45.

d. **Nutritional composition of pellet:** In response to reviewer's request we have added an additional statement in method on page 11 (revised manuscript) indicating that nutritional composition of pellet consisted of 52.72% carbohydrate, 19.85% protein, 2.41% fat, 4.27% fiber, 1.05% calcium, 13.24% moisture, 5.82% ash, 0.64% phosphorus, and 3.28 kcal /gm.

PURINA RODENT CHOW ANALYSIS RESULT

ASSAY	ANALYSIS	UNIT
<i>NUTRIENTS</i>		
MOISTURE(OVEN)	13.24	%
CARBOHYDRATE	52.72	%
PROTEIN(PROTEIN ANALYZER)	19.85	%
FAT(ACID HYDROLYSIS)	2.41	%
FIBER(FIBERTEC)	4.27	%
ASH(FURNACE)	5.82	%
CALCIUM(AAS)	1.05	%
PHOSPHORUS(COLOMETRIC)	0.64	%
<i>HEAVY METALS</i>		
As (ICP)	0.2	ppm
Cd(ICP)	Not Detected	ppm
Hg(MERCURY ANALYZER)	1.3	ppb
Pb(ICP)	Not Detected	ppm
Se(ICP)	0.66	ppm
<i>AFLATOXIN(ELISA)</i>		
B1, B2, G1, G2	0.10	ppb
<i>CHLORINATED HYDROCARBON</i>		
DDT(GC)	0.50	ppm
<i>ORGANOPHOSPHATES</i>		
MALATHION(GC)	8.00	ppm
<i>Microbial Tests</i>		
Salmonella	Negative	mm
Total Bacteria	1.0x10 ⁶	cfu/g
E.Coli	Negative	
<i>PHYSICAL PROPERTIES</i>		
PELLET SIZE(DxL)	15.4x13.4	mm
PELLET COLOR	Dark Brown	-
HARDNESS	More than 20	kg/cm ²

e. **How dietary intake of the UndN group was determined ?:** Dietary intake of the UndN group was based on daily dietary intake of the S rats. Each of the S rats was housed in an individual cage allowing for measurement of nutrient intake. The one- week intake of 46g consumed by the S group was divided by 7 for a daily average of 6.6 to 6.7 g. This amount was offered to the UndN group who consumed the entire amount daily.

f. **Were the rats kept in metabolic cage?:** No, they were not kept in metabolic cages.

g. **Where rats kept in individual cages or where more than 1 rat;** Rats were housed in individual cages.

h. **“Intake of water and Diet- How was this done? on page 10 line 13-16:** See response to e above.

5. **Data analysis; Suggest doing a more powerful statistical procedure, ANOVA and Duncan's procedure:** In response to reviewer's comments, we have analyzed our data by ANOVA and post-hoc multiple pair-wise comparisons using Duncan's procedure.
6. **References:** In response to reviewer's request we have eliminated some of references quite dated. These are; Chokroverty et al., 1976; Axelsson et al., 1984; Nam, 1964; Essen, Fohlin, Thoren, & Saltin, 1981. In the case of - Brooke & Kaiser (1970), although dated the methods outlined in this paper continue to be used and thus we retained this reference.
7. **Tables;**
 - a. **Table 1: post body organ/muscle tissue weights for the 3 groups:** Body organ weights were not obtained. We feel that the addition of muscle weights to this table would make the presentation less clear. However, if the editor agrees with this comment, we can make the change.
 - b. **Table 2: were rats housed in separate or group cages?:** Yes, rats were housed in separate cages. **why wasn't caloric intake determined?:** Because we believed that determination of diet intake would be enough to accomplish the purpose of our study. We chose to report in gms rather than calories, however this calculation can be done since the food contained- 3.28kcal/gm.
 - c. **Rounding all numbers in the table to whole numbers:** For example, 89.9 ± 16.7 to 90 ± 17 , 204.1 ± 50.9 to 205 ± 51
Because of the precision of our instruments we feel comfortable in retaining the numbers to the tenth as presented. We reviewed other physiologically based Nursing Research papers and noted their use of data to the tenth. Fontana, J.A., 49(2):91-96, 2000, Bliss et al., 50(4):203-213, 2001.

Reviewer 5

1. **Problem statement:** In response to the reviewer's suggestion we have added three hypotheses. See response to Reviewer#1, question #2.
2. **Literature review:**
 - a. **Give information the difference between Type I and II muscle:** In response to reviewer's suggestion, we have added statements to indicate that type I fibers are high in myoglobin and oxidative enzymes, have many mitochondria in keeping with their ability to perform tonic contraction, and have low myosin ATPase activity and slow speed of contraction. Type II fibers are rich in glycolytic enzymes and are involved in rapid phasic contraction and have higher myosin ATPase activity than type I fibers resulting in fast contraction.(see revised manuscript page 6).
 - b. **Why both muscle types should be examined:** Previous studies which found selective type II fiber atrophy examined patients one month or more post stroke. Our question was whether examination seven days after denervation would reveal greater differences between type I and II fibers.
3. **Methods:**
 - a. **Number of animals:** In response to reviewer's comments, we described both the initial number and the reasons for the final number reflected in the statement on page 9 and 10 of revised manuscript.
 - b. **Data analysis:** In response to reviewer's comments, we have analyzed our data by ANOVA and Duncan's procedure as a post-hoc test.
4. **Results:**
 - a. **If there is no difference between groups please say so right up front:** In accordance with reviewer's comments, the manuscript has been revised.

b. **Presented in graphs rather than tables 4-7:** In accordance with reviewer's comments, our data have been presented in graphs. We have made bar graphs of table 3 (muscle weights), table 4 (cross-sectional area of soleus muscle) and table 7 (myofibrillar protein content) to illustrate the difference. (see figure 2, 3 and 4 revised manuscript)

5. **Discussion: please address the issue of the under fed groups since one of the overall aims of the study was to determine if similar changes observed in stroke could be produced by undernutrition alone. P 17 line 13-14. Why no differences were noted between controls and the underfed animals? Is it possible that you need more animals in the food-deprived group to detect differences?;** We reanalyzed our data and found that differences were present between the controls and the underfed rats.
6. **On page 19, 17: This statement is rather confusing and not necessarily supported by your data since the underfed was not statistically significant than controls. How do you know that undernutrition contributes to atrophy based on the results of this study:** Results from the reanalysis demonstrated differences between the underfed rats and the controls.