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The effects of exercise and conjugated linoleic acid intake on IGF-1 and pro-inflammatory cytokines in atrophied skeletal muscle of rats

*Sanghyun Kim, Kijin Kim**

Department of Physical Education, Keimyung University, Daegu, Korea

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ABSTRACT

Background: Conjugated linoleic acid (CLA) can be proposed as an effective nutrient for skeletal muscle atrophy. However, the research related to this is still insufficient. This study was carried out to analyze the mRNA expression of IGF-1 and cytokines in atrophied skeletal muscle of rats.

Methods: Forty-two rats were randomly divided into seven groups, each group containing six rats. Sham-Pre and USN-Pre groups underwent a sham operation and a unilateral sciatic nerve (USN) cut, and were sacrificed 1 week later. Other groups had 4 weeks of treatment exercise and CLA intake, and then their blood, liver, and skeletal muscles were sampled after sacrifice.

Results: Among the treatment groups, the group treated with both exercise and CLA (USN-EC) showed the lowest body weight. Groups with the sciatic nerve cut showed significantly ($p < 0.05$) lower muscle weight than groups with the sham operation. However, exercise and CLA intake had no effect on muscle weight. Regarding IGF-1 mRNA, the USN-EC group showed significantly higher expressions in the red muscle of the gastrocnemius and liver than the Sham-Pre and USN-CLA groups. Regarding TNF-α pro-inflammatory cytokine, there was no particular trend; however, the expression of IL-1β mRNA increased in the white muscle of the gastrocnemius muscle and tibialis anterior muscle after sciatic nerve cut, but showed a decrease with exercise and CLA treatment. Particularly in the gastrocnemius white muscle, the group treated with both exercise and CLA showed a significant decrease as compared to groups without treatment after sciatic nerve cut so that positive effects can be expected.

Conclusion: It is thought that combining treadmill training with CLA partially influences pro-inflammatory cytokines, so that this can act positively on improving skeletal muscle atrophy caused by sciatic nerve cut.

* *Corresponding author*. Department of Physical Education, Keimyung University, 1000 Shindang-dong, Dalseo-gu, Daegu, 704-701, Korea. E-mail address: kjk744@kmu.ac.kr (K. Kim).

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1. Introduction

Muscle fibers are controlled by the motor nerves, which have an important effect on changing the shape and function of skeletal muscle.[1] Therefore, damage or removal of the motor nerves controlling skeletal muscle influences the muscle's target tissue of peripheral neurons.[2] If neural innervation toward the muscle is blocked by neural damage, the contraction function of skeletal muscle is lost. Muscle mass then decreases, and muscle atrophy occurs due to the diminished amount of natural activity and motion.[1] Muscle atrophy is defined as a decrease in muscle mass. In addition, muscle fiber composition and the ratio of protein synthesis and decomposition changes.[3,4] These changes are adjusted by interaction between elements related to assimilation and catabolism. In particular, it is known that IGF-1 participates in the growth and development of tissue[5], as well as its regeneration.[6] Also, it multiplies myoblasts, promotes differentiation of muscle cells, and plays an important role in developing muscle by acting on growth of cartilage, and increasing cell differentiation.[7] Pro-inflammatory cytokine expression is an important mechanism of myopathy, and increased cytokines due to sepsis and chronic heart failure can be determined from serum and skeletal muscle tissue.[8,9] TNF-α, among various cytokines, restrains the synthesis of MyoD, a key controller of muscle formation, by activation of NF-κB.[10] According to recent research, TNF-α is known to restrict muscle formation through activation of caspases.[11] IL-1 is also known to control skeletal muscle mass through restraining MyoD by activation of NF-κB, or increasing protein decomposition by raising MuRF1.[12]

Electrical stimulation[13,14] and exercise[15,16] are used to regain use of atrophied skeletal muscle. External electrical stimulation has not been widely used to attempt muscle stimulation, due to its risks during treatment, or difficulties in use.[17] However, a therapeutic approach using exercise has been frequently applied. Since Vecchi first used exercise therapy by applying treadmill training to mice with neural damage in 1929, many research efforts on recovering function by exercise have been carried out.[16] However, there is room for controversy because the intensity and duration of exercise for comparison of effects of exercise on denervated muscle were not standardized.[16,18,19] Besides exercise, the importance of nutritional status cannot be overlooked. This is because the ratio of protein synthesis and decomposition relies on nutritional status.[20] There is little research regarding the effects of nutritional status as a treatment for atrophied skeletal muscle after neural damage. Therefore, proposing effective nutrients is considered important to prevent muscle atrophy by denervation. Conjugated linoleic acid (CLA), among various nutrients, has been concluded to be effective in preventing muscle atrophy. In the research related to anti-obesity activity, among various physiological effects, Blankson et al[21] and Park et al[22] reported that it increases lean body mass. Therefore, it is considered that CLA can be proposed as an effective nutrient for skeletal muscle atrophy. However, research related to this matter is still insufficient. Therefore, this study was carried out to analyze the expression of IGF-1 and mRNA of pro-inflammatory cytokines in atrophied skeletal muscle of rats. They were treated with exercise and CLA intake for 4 weeks after their sciatic nerve was cut, in order to examine the effects of exercise and CLA intake in atrophied skeletal muscle.

2. Methods

2.1. Experimental design

This study was approved by the Institutional Animal Care and Use Committee of the Medical School of Keimyung University (receipt number: KM-08-01). All rats received adjustment training on a treadmill after having a 1-week acclimation period. After adjustment training, their sciatic nerve was cut. Following a 1-week recovery period, a program of treadmill training and CLA intake was implemented. Before and after the 4-week program, each group was sacrificed and then samples were collected (Fig. 1).

Groups of rats were divided into a sham operation (Sham)-group and a unilateral sciatic nerve cut (USN)-group. Sham groups were again divided into a Pre (pre-sacrifice) group and a NT (non-treatment) group, and USN groups were divided into Pre, NT, Ex (exercise), CLA (CLA intake), and EC (exercise + CLA) groups.

2.2. Laboratory animals

This study used 42 male SD rats which were 4 weeks old (Hyochang Science, Daegu, Korea); these were divided into seven groups of six rats. Due to the death of one rat from each of the Sham-Pre, USN-Pre, USN-CLA, and USN-EC groups, 38 rats completed the experiment.

Light and dark cycles were 12 hours each per day. The temperature and relative humidity were 24 ± 1°C and ~60%, respectively. Three rats were housed per cage. All rats had free access to water and food.

2.3. Sciatic nerve cut

All rats had a 1-week adjustment period in preliminary housing, and then the sham operation or sciatic nerve cut was implemented. Thirty rats among the total of 42 had a right sciatic nerve cut, according to the method of Baumann et al[23] and Furuno et al.[4] The remaining 12 rats with no sciatic nerve cut had the same procedure as the other rats, but were stitched up without damage to the sciatic nerve. These processes were carried out in aseptic conditions.

2.4. Treadmill running and CLA intake

The capability of rats to exercise 1 week after surgery was verified using the treadmill running protocol of van Meeteren et al[19] with modifications (15 m/minute, inclination 3~5%, 25~30 minutes/day, 5 days/week). Liquid type CLA (HK Biotech Co., Ltd., Jinju, Korea) was given and the content of CLA is presented in Table 1.

2.5. Sample preparation

Using a Dial-O-Gram Balance (OHAUS, Parsippany, NJ, USA), the body weight of the rats was measured weekly.

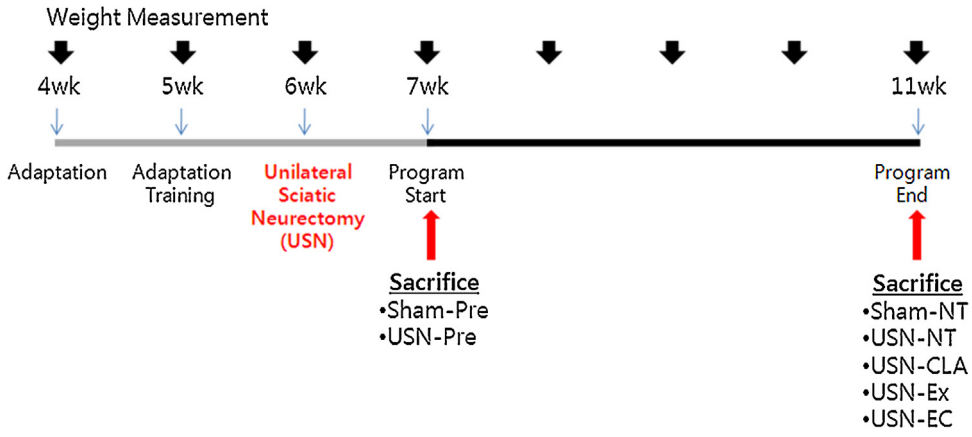

Fig. 1 – Overall procedure for experiment.

Table 1 – Composition of CLA.

Item	Element standard	Content
CLA content (%)	70~80%	76.98%
c-9, t-11 CLA (%)	>35%	36.89%
t-10, c-12 CLA (%)	>35%	37.15%
c-9, c-11 CLA (%)	No	1.90%
t-9, t-11 CLA (%)	<3.0%	1.02%
Peroxide value (meq/kg)	<3.0	1.20 ± 0.04

CLA, conjugated linoleic acid

Table 2 – Primer sequences used for IGF-1 and cytokines real-time PCR assay.

Gene	Primer Sequences
IGF-1	Forward 5 -GGGTGTTGTAGACATTCGGTTGC-3
	Reverse 5 -GCTACATAGGAAGAAATGGAGATAAGG-3
IL-1β	Forward 5 -GCTGACAGACCCCAAAAGAT-3
	Reverse 5 -AGCTGGATGCTCTCATCTGG-3
TNF-α	Forward 5 -AGAACTCCAGGCGGTGTCT-3
	Reverse 5 -GAGCCCATTTGGGAACTTCT-3
18S rRNA	Forward 5 -ATCCATTGGAGGGCAAGTCT-3
	Reverse 5 -AACTGCAGCAACTTTAATATACGC-3

The blood of the rats sacrificed before or after the program was taken from inferior vena cava for IGF-1 concentration. The blood was separated using a centrifuge (Combi-514R, Hanil Science Industrial, Incheon, Korea) with 3500 rpm at 4°C for 15 minutes, and then the separated serum was kept at –70°C until analysis.

After taking blood, saline was injected into the portal vein using a 50 mL syringe, and then the liver was harvested. It was quickly frozen in liquid nitrogen and stored at –70°C. The gastrocnemius muscle (GA), soleus muscle, and tibialis anterior (TA) muscle were harvested. The weight of each was measured using an electronic scale (EPG213, Ohaus Co., Parsippany,NJ, USA). The gastrocnemius muscle was divided into white muscle (WG) and red muscle (RG), and then was quickly frozen in liquid nitrogen and stored at –70°C until analysis.

2.6. *ELISA*

The process of measuring IGF-1 concentration in the blood was carried out using a mouse/rat IGF-1 enzyme-linked immunosorbent assay (ELISA) kit (DSL-10-29200, Diagnostic Systems Laboratories Inc., Webster, TX, USA) according to the analysis method of the manual included in the kit, and then the concentration was measured using a Microplate Reader (ELx800, BioTek Inc., Winooski, VT, USA) at 450 nm.

2.7. *mRNA expression*

Total RNA was extracted from tissue of liver and muscle (gastrocnemius and tibialis anterior muscle) using TRI reagent (Sigma-Aldrich, Inc., St. Louis, MO, USA), and the concentration was measured by using a UV spectrophotometer at 260 nm (UV-mini 1240, Shimadzu Co., Kyoto, Japan). RNA was cleaned using TURBO DNA-free Kit (Ambion Inc., Austin, TX, USA). The extracted RNA synthesized cDNA using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). For the analysis of mRNA expression, real time PCR was carried out in iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, USA). The mRNA expression level of IGF-1, TNF-α, and IL-1β was quantified. (Table 2)

2.8. *Statistics*

All data are expressed as mean ± SD and analyzed by one-way ANOVA using the SPSS for Windows (ver. 12.0). When a significant main effect was observed, *post hoc* analysis (Tukey's) was performed to locate the difference. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. *Body weight*

Regarding changes in body weight (BW) of rats during the experiment, all groups had the same trend of BW change from after the sciatic nerve cut until just before treatment. During the 4-week treatment, the Sham-NT group showed the highest weight increase, and the USN-NT group showed a lower BW increase. USN-CLA, USN-EX, and USN-EC groups that had treatment showed a lower BW increase than the control group, and the USN-EC group that had both exercise and CLA

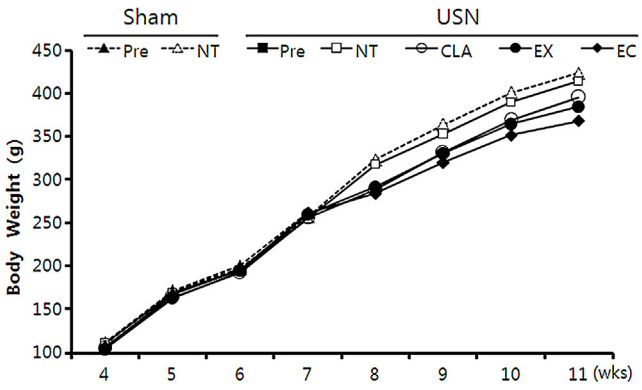

Fig. 2 – Comparisons of body weight among groups. CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; NT, non-treatment; Pre, pre-sacrifice; Sham, sham operation; USN, unilateral sciatic neurectomy.

presented the lowest BW increase among groups with treatment (Fig. 2).

3.2. *Skeletal muscle weight*

Fig. 3 shows the comparison between right and left muscle of the USN-NT group having no treatment during the 4 weeks. As shown in Fig. 3, it was verified that the right muscle with sciatic nerve cut shrank remarkably as compared to the left muscle without sciatic nerve cut.

Regarding the change in skeletal muscle weight during the 4-week treatment after sciatic nerve cut (Table 3), the USN-NT, USN-CLA, USN-EX, and USN-EC groups showed significantly lower weight ($p < 0.001$) than the Sham-Pre, USN-Pre, and Sham-NT groups in gastrocnemius muscle and tibialis anterior muscle. In the soleus muscle, USN-NT, USN-CLA, and USN-EX groups showed significantly lower weight ($p < 0.001$) than Sham-Pre, USN-Pre, and Sham-NT groups. The USN-EC group showed significantly lower weight ($p < 0.001$) than the Sham-Pre and Sham-NT groups, and the USN-Pre group showed significantly lower weight ($p < 0.01$) than the Sham-Pre and Sham-NT groups. In the group comparison to analyze the effects of exercise and CLA treatment, there was no significant difference in all tissues. However, the USN-EX group (tibialis anterior muscle) and the USN-EC group (soleus muscle) showed a slightly higher weights after the treatment.

Table 3 – Comparisons of skeletal muscle weight among groups (mg/g body weight).

Group		GA	TA	Soleus
Sham	Pre	3.99 ± 0.97	1.22 ± 0.27	0.29 ± 0.07
	NT	4.26 ± 1.09	1.32 ± 0.33	0.35 ± 0.08
USN	Pre	3.46 ± 0.27	0.99 ± 0.08	0.18 ± 0.06*
	NT	1.06 ± 0.12*†	0.36 ± 0.05*†	0.08 ± 0.02*†
	CLA	1.02 ± 0.09*†	0.34 ± 0.04*†	0.08 ± 0.01*†
	Ex	1.08 ± 0.10*†	0.54 ± 0.35*†	0.08 ± 0.01*†
	EC	1.04 ± 0.10*†	0.40 ± 0.04*†	0.09 ± 0.02*

Values are means ± SD.
CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; GA, gastrocnemius; NT, non-treatment; Pre, pre-sacrifice; Sham, sham operation; TA, tibialis anterior; USN, unilateral sciatic neurectomy.
* Significantly different from Sham-Pre and Sham-NT.
† Significantly different from USN-Pre.

3.3. *Circulating levels of IGF-1*

IGF-1 levels, following the 4-week treatment after sciatic nerve cut, did not have significant differences among groups, as shown in Table 4. However, the USN-Pre and USN-NT groups without treatment after sciatic nerve cut showed generally low IGF-1 levels. The group with CLA and exercise treatment showed similar levels of IGF-1 to the Sham-Pre and Sham-NT groups, and showed levels that were a little higher than the USN-Pre group.

3.4. *IGF-1 mRNA expression*

The results of comparing the trends of IGF-1 mRNA expression in liver and skeletal muscle following the 4-week treatment after sciatic nerve cut are shown in Table 5. There were significant differences ($p < 0.05$) among groups in liver, WG and RG. From the *post hoc* test results, the USN-EC group showed significantly higher IGF-1 mRNA expression ($p < 0.05$) than the Sham-Pre group. In the RG, the USN-EC group showed significantly higher expression ($p < 0.05$) than the USN-CLA group. However, there was no significant difference among groups in the WG.

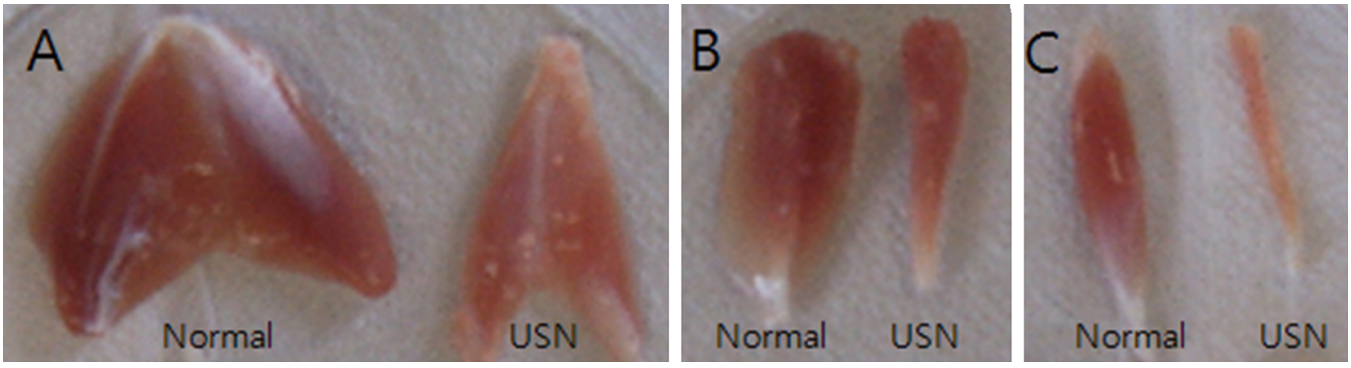

Fig. 3 – Diagrams of normal and sciatic neurectomy muscle. (A) Gastrocnemius; (B) tibialis anterior; (C) soleus. USN, unilateral sciatic neurectomy.

Table 4 – Comparisons of serum IGF-1 concentration among groups (ng/mL).

GroupItem	Sham		USN				
	Pre	NT	Pre	NT	CLA	Ex	EC
IGF-1	1594.9 ± 99.2	1625.3 ± 227.8	1499.1 ± 192.5	1490.9 ± 229.1	1654.0 ± 120.3	1575.5 ± 82.6	1572.5 ± 132.5

CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; NT, non-treatment; Pre, pre-sacrifice; Sham, sham operation; USN, unilateral sciatic neurectomy.

3.5. *TNF-α mRNA expression*

The results of comparing the trends of TNF-α mRNA expression in skeletal muscle following the 4-week treatment after sciatic nerve cut are shown in Table 6. There was no significant difference among groups. In the white and red muscle of gastrocnemius muscle, there was no significant difference found between USN-NT group and the group with exercise and CLA treatment. However, in the TA, groups with treatment showed lower trends in TNF-α mRNA expression than the USN-Pre and USN-NT groups.

3.6. *IL-1β mRNA expression*

The results of comparing the trends of IL-1β mRNA expression in skeletal muscle following the 4-week treatment after sciatic nerve cut are shown in Table 7. There was a significant difference in IL-1β mRNA expression among groups in white muscle ($p < 0.05$) and red muscle ($p < 0.01$) of gastrocnemius muscle, and tibialis anterior muscle ($p < 0.05$). From the results of *post hoc* testing, the USN-NT group showed significantly higher expression ($p < 0.05$) than the Sham-Pre, Sham-NT, and USN-EC groups in white muscle of gastrocnemius muscle, and remarkably higher trends in expression than USN-Pre ($p = 0.055$) and USN-Ex ($p = 0.075$) groups. The USN-EC group showed significantly higher expression ($p < 0.05$) than the Sham-Pre and Sham-NT groups in the red muscle of gastrocnemius muscle, and the USN-NT group presented significantly higher expression ($p < 0.01$) than the Sham-Pre group in tibialis anterior muscle.

Table 5 – Expression of IGF-1 mRNA in tissues among groups (arbitrary units).

Group		Liver	WG	RG	TA
Sham	Pre	0.50 ± 0.19	2.02 ± 0.97	1.89 ± 0.79	1.92 ± 1.30
	NT	0.99 ± 0.43	1.16 ± 0.30	1.53 ± 0.23	1.36 ± 0.42
USN	Pre	0.64 ± 0.13	1.89 ± 0.34	2.25 ± 0.51	1.85 ± 1.27
	NT	0.95 ± 0.25	3.97 ± 2.64	2.70 ± 1.00	1.11 ± 0.29
	CLA	0.92 ± 0.09	3.51 ± 1.55	1.40 ± 0.35	2.38 ± 0.65
	Ex	0.65 ± 0.17	3.45 ± 2.20	1.58 ± 0.54	1.69 ± 0.51
	EC	1.08 ± 0.47*	3.20 ± 1.27	2.86 ± 1.28†	2.07 ± 0.96

Values are means ± SD.
CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; NT, non-treatment; Pre, pre-sacrifice; RG, red gastrocnemius; Sham, sham operation; TA, tibialis anterior; USN, unilateral sciatic neurectomy; WG, white gastrocnemius.
* Significantly different from Sham-Pre.
† Significantly different from USN-CLA.

Table 6 – Expression of TNF-α mRNA in tissues among groups (arbitrary units).

Group		WG	RG	TA
Sham	Pre	2.08 ± 1.39	0.86 ± 0.34	0.95 ± 0.53
	NT	0.93 ± 0.43	1.14 ± 0.45	1.20 ± 0.42
USN	Pre	1.00 ± 0.34	1.11 ± 0.36	1.23 ± 0.63
	NT	1.63 ± 0.26	0.88 ± 0.33	1.05 ± 0.50
	CLA	1.84 ± 0.87	0.78 ± 0.20	0.71 ± 0.37
	Ex	1.69 ± 1.42	0.67 ± 0.40	0.86 ± 0.20
	EC	1.82 ± 1.12	1.16 ± 0.40	0.98 ± 0.30

Values are means ± SD.
CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; NT, non-treatment; Pre, pre-sacrifice; RG, red gastrocnemius; Sham, sham operation; TA, tibialis anterior; USN, unilateral sciatic neurectomy; WG, white gastrocnemius.

Table 7 – Expression of IL-1β mRNA in tissues among groups (arbitrary unit).

Group		WG	RG	TA
Sham	Pre	1.69 ± 1.32	1.10 ± 0.57	0.48 ± 0.15
	NT	0.82 ± 0.29	1.91 ± 0.52	1.27 ± 0.68
USN	Pre	2.50 ± 0.51	3.77 ± 1.71	1.32 ± 0.57
	NT	10.23 ± 8.58*	3.53 ± 2.08	2.13 ± 1.12†
	CLA	5.59 ± 5.71	3.49 ± 1.83	1.02 ± 0.47
	Ex	3.15 ± 2.21	2.86 ± 1.68	0.99 ± 0.66
	EC	2.18 ± 1.24‡	5.70 ± 2.68*	1.12 ± 0.40

Values are means ± SD.
CLA, conjugated linoleic acid intake; EC, treadmill exercise + CLA intake; Ex, treadmill exercise; NT, non-treatment; Pre, pre-sacrifice; RG, red gastrocnemius; Sham, sham operation; TA, tibialis anterior; USN, unilateral sciatic neurectomy; WG, white gastrocnemius.
*Significantly different from Sham-Pre and Sham-NT.
† Significantly different from Sham-Pre.
‡ Significantly different from USN-NT.

4. Discussion

4.1. *Reduced skeletal muscle weight after sciatic nerve cut*

Hyatt et al[1] reported that if neural innervation toward muscle is blocked by neural damage, the contraction function of skeletal muscle is lost. Muscle mass is decreased and muscle atrophy occurs due to the diminished amount of natural activity and motion. Midrio[24] reported that innervation of denervated skeletal muscle incurs muscle atrophy featured by a reduction of total muscle mass and thickness of muscle fiber. Also, this study assumed that innervation of denervated skeletal muscle by sciatic nerve cut decreases skeletal muscle mass, as shown in Table 3 and Figure 3. In the many preceding studies done by Beris et al,[25] van Meeteren et al,[16] and others, the effect of exercise treatment after neural damage was

positively estimated by excessive growth of axons, increased number and diameters of axons, increased amounts of creatine inside muscles, and increased muscle weight. In this study, however, groups without treatment and groups with treatment after sciatic nerve cut had similar trends in all skeletal muscle weight. This concurs with the opinion of Herbison et al[26] that exercise after neural damage was not effective because there was no difference between muscle weight and the diameter of muscle fiber. This study carried out an exercise protocol by modifying and supplementing exercise protocol proposed by van Meeteren et al.[19] It is a little lower in intensity and shorter in duration than those applicable to VO_2max 60% reported by Taylor et al[27] regarding the positive result on skeletal muscle mass. Thus, the applied exercise intensity and duration of this study might be insufficient to have an effect. When neural damage is noticeable or a nerve is cut, it is known that keeping neural continuity by early operation is the most important thing.[28] This study had difficulty in providing an accurate decision because it did not implement various kinds of methods for estimating the recovery level of exercise function. Considering the movement during treadmill training (not measured), keeping neural continuity was not sufficient. Therefore, it is considered that enough stimulation for recovering skeletal muscle mass could not be made. According to many preceding research efforts, the effect that CLA increases skeletal muscle mass is not definite. It was reported that the effect of CLA on muscle mass differs according to isomers. Park et al[22] reported that a group taking *trans*-10, *cis*-12 CLA isomer had increased contents of body protein, water, and ash. In recent research, however, Lee et al[29] reported that *cis*-9, *trans*-11 CLA stimulated proliferation and differentiation of C2C12 skeletal muscle cells, but *trans*-10, *cis*-12 CLA restrained it. The effect of CLA on skeletal muscle differs depending on the isomer, and the effects have not been exactly proved to date. Therefore, further research regarding the effects of various CLA isomers on muscle mass should be carried out.

4.2. *IGF-1 levels and mRNA in the blood and tissues*

It is known that IGF influences organogenesis regarding regeneration of damaged muscle and recovery of atrophied muscle by involving a proliferation of muscle stem cells, migration of myogenic precursor cells, and differentiation of myotubes.[30] The research, however, regarding the effect of IGF-1 on recovery of skeletal muscle is insufficient when exercise treatment is applied to skeletal muscle that has atrophied due to denervation. Therefore, this study examined whether treadmill running and CLA intake for 4 weeks after sciatic nerve cut increased the IGF-1 in the blood and tissue. We showed that IGF-1 levels in the blood and mRNA expression in tissues did not show significant changes from with regard to treadmill running and CLA intake. The synthesis and secretion of IGF-1 is mainly controlled by growth hormone. When growth hormone is secreted in the pituitary gland, growth hormone receptor in the liver promotes IGF-1 synthesis, and the IGF-1 produced is distributed throughout the body via the bloodstream, and biochemical reactions including assimilation of skeletal muscle are promoted. It is known that this reaction can be activated by exercise. Kanaley et al[31] reported that stimulation of growth hormones increased after treadmill running for 16 weeks with 70% VO_2max strength. Nguyen et al[32] reported that the level of IGF-1 showed a decrease or no difference in cases of exercising with adequate strength and long periods. Also, de Rezende Gomes et al[33] reported that IGF-1 levels showed differences according to the intensity and period of exercise. Therefore, the results of this study can be more clearly explained if further research to examine change trends of IGF-1 levels according to the exercise intensity were carried out.

A hypothesis was proposed that CLA takes effect by controlling various growth factors,[34] but Pariza et al[35] reported that it is not clear what relationship CLA has with growth factors participating in assimilation. Kim et al,[36] who investigated the relationship between CLA and IGF-1, reported that CLA decreased the level of IGF-1 receptor mediating IGF-1 action using human colon cancer cells. Li et al[37] reported that the IGF-1 and IGF binding proteins (IGFBP) are decreased by CLA in bone metabolism. Kelly et al[38] reported that CLA did not influence the IGF-1 level. The cause has not been clarified until now, however. Pariza et al,[35] Park et al,[22] and Turpeinen et al[39] reported that body protein and inflammatory response presented different effects depending on the amount of CLA or which isomer is present. Therefore, it is considered that IGF-1 acting positively on skeletal muscle has the possibility of showing a different response. In liver and red muscle of gastrocnemius muscle, the USN-EC group showed a significantly higher response than the Sham-Pre group and group with only CLA treatment, so that more positive results can be expected in cases of combining exercise and CLA treatment. In the white muscle of gastrocnemius muscle, the USN-CLA, USN-Ex, and USN-EC groups showed lower results than the USN-NT group so that the difference by tissue was shown. Therefore, a study comparing the differences by types of muscle fiber is needed.

As mentioned above, there is a lack of studies on the relationship of CLA to IGF-1 acting positively on skeletal muscle. Also, the effect of CLA on IGF-1 acting on skeletal muscle is not clearly known,[35] so further research dealing with this should be carried out. From this point of view, this study is considered important in terms of analyzing the relationship between IGF-1 of atrophied skeletal muscle and CLA.

4.3. *Expression of TNF-α and IL-1β*

Muscle protein synthesis occurs according to the positive action of IGF-1,[40] and it is known that decreased IGF-1 levels are related to reaction by pro-inflammatory cytokines.[41] With regard to this, Broussard et al,[42] mentioned that IL-1β weakened the differentiation of skeletal muscle cells by IGF-1, and downstream signaling by IGF-1 receptor in skeletal muscle cells. Also, Broussard et al,[42] reported that IGF-1 resistance, such as decreasing protein synthesis, occurred, in cases of incubating skeletal muscle cells with pro-inflammatory cytokine including both TNF-α and IL-1β. As we have shown, it is considered that there was a possibility of not having normal action of IGF-1 due to increased pro-inflammatory cytokine. However, IGF-1 resistance caused by inflammatory response of skeletal muscle was not clearly revealed at molecular level[42] so that further research related to this is needed.

In the case of muscle damage, various cytokines, such as IL-1α, IL-1β, IL-2, IL-4, IL-6, and TNF-α, are expressed or secreted from inflammatory cells, vascular endothelial cells, and muscle cells.[43,44] In particular, TNF-α, as well as IL-1, show changes in secretion influenced by immunocytes including the inflammatory response,[45] and is known to cause biochemical changes in various cells such as catabolism of skeletal muscle, and so on.[46] In this study, the trend of TNF-α was not clearly verified. However, the group with exercise and CLA treatment showed significant results and trends of IL-1β in the white muscle of gastrocnemius muscle and tibialis anterior muscle. The group with exercise and CLA treatment showed rather higher expression in red muscle of gastrocnemius muscle. Therefore, it is thought that for pro-inflammatory cytokines, research dealing with differences by types of muscle fiber like IGF-1 is needed.

Most cells in the human body have a receptor toward TNF-α so that various kinds of cells, such as skeletal muscle cells, neurons, and so on, can produce TNF-α.[46] Pro-inflammatory stimulation like TNF-α increases COX2 (cyclooxygenase-2) enzyme, and produces PGE2 (prostaglandin E2) pro-inflammatory material.[47] PGE2 is produced by macrophages producing IL-1 and TNF,[48] and increases the decomposition of muscle when incubated with skeletal muscle cells. For this reason, IL-1 was known to act on muscle decomposition by inducing PGE2 release.[37,49] It is considered that reducing COX2 and PGE2 can act positively on skeletal muscle atrophy because this study also showed a decrease in IL-1β mRNA by treatment with exercise and CLA. However, the accurate mechanism of this is not known. The reason is that pro-inflammatory cytokines are known to react through various pathways. Zamir et al[50] reported that TNF-α participates in decomposition of muscle protein by glucocorticoids, and IL-1 decomposes muscle protein by a different mechanism from TNF-α. Remels et al[40] mentioned that inhibition of muscle formation by TNF-α and IL-1β restrains MyoD synthesis, a key controller of muscle formation through the activation of NF-κB. Therefore, if further research for revealing the downstream mechanism of TNF-α and IL-1β with regard to exercise and CLA treatment is carried out, it will help find the causes of different trends shown in TNF-α and IL-1β, and not increased skeletal muscle weight by decreased IL-1β mRNA.

5. Summary and Conclusion

This study was carried out using treadmill running, CLA, or both treadmill and CLA as treatments for muscle atrophy resulting from a sciatic nerve cut. The expression levels of IGF-1 mRNA, and mRNA of TNF-α and IL-1β pro-inflammatory cytokines in skeletal muscle was examined. The group with exercise had slightly higher skeletal muscle weight; however, this was not significant. For a more noticeable effect, further research that considers the intensity of treadmill training and amount of CLA, and physiological activities according to different isomers, in order to reveal the downstream mechanism of TNF-α and IL-1β with regard to exercise and CLA treatment, should be continuously implemented. In the factor analysis related to assimilation and catabolism in skeletal muscle, there was no significant change in IGF-1. However, in the change of cytokines, the USN-EC group showed a significant decrease in IL-1β mRNA in the white muscle of gastrocnemius muscle as compared to the USN-NT group. Therefore, it is thought that combining treadmill training with CLA partially influences pro-inflammatory cytokines, so that this can act positively on improving skeletal muscle atrophy caused by sciatic nerve cut.

Conflicts of interest

All authors have no conflicts of interest to declare.

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