INTRODUCTION
Muscle atrophy makes it difficult to return to normal life after fracture or surgery. Study of muscle atrophy after injury, fracture fixation, joint surgery, spine surgery, and so on were few. It is important for orthopaedic surgeon to recover muscle atrophy of patients.

Previously, we demonstrated that transcutaneous application of CO$_2$ up-regulated O$_2$ pressure in the local tissue. Additional we induced that transcutaneous CO$_2$ application to the lower limbs increased expression of PGC-1$\alpha$, VEGF and SIRT1, increased numbers of mitochondria, and changed from IIB fiber to IIA fiber, as similar to change after exercise. This study indicated that transcutaneous CO$_2$ application could cause similar effect to the exercise in skeletal muscle. However, the effect of transcutaneous CO$_2$ application on skeletal muscle atrophy after fracture has not been investigated.

The aim of this study is to investigate the change of mitochondria and gene expression of the lower limbs muscle (tibialis anterior muscle: TA and soleus muscle: SOL) after femoral shaft fracture.

MATERIALS AND METHODS
Femoral Fracture in animal model: Ten week old male Sprague–Dawley rats were used in this study. All animal procedures were performed under the approval and guidance of the Animal Care and Use Committee at the authors’ institution. Briefly, a 1.2 mm diameter K-wire was inserted retrograde into the right femoral intramedullary canal and a closed transverse femoral shaft fracture was produced in all animals using a three-point bending apparatus with a drop weight. Unprotected weight bearing was allowed postoperatively. For gene expression analysis, six animals from each group were euthanized at the following time points: prefracture (day 0) and postfracture days 7, 14, and 21.

Procedure for CO$_2$ treatment: Under pentobarbital anesthesia (6 ml/kg), we cut the hairs of right lower limbs, and applied hydrogel to these lower limbs. The CO$_2$ adaptor was put on limbs, and CO$_2$ was administrated into the adaptor for 20 minutes. This treatment was performed five times a week as describe previously. Muscle preparation: The rats were sacrificed by an overdose pentobarbital anesthesia followed by decapitation, and intact TA and SOL were dissected. The muscles were weighed after excess connective tissue, was removed and then immediately frozen as describe previously.

Gene analysis: Total RNA was extracted in TRIzol reagent and RNeasy Mini Kit. The first-strand cDNA was synthesized (100 ng total RNA) using High Capacity cDNA Transcription Kit. cDNA was performed by Real-time PCR, as described previously. Gene expression of atrophy-related gene (FOXO-1, Atrogin-1) and synthesis-related gene (PGC-1$\alpha$, VEGF and IGF-1) were measured using real-time PCR in SOL as describe previously. Genomic DNA was isolated from a 10 mg transversal slice of medially TA using the GenElute Mammalian Genomic DNA Miniprep Kit. Mitochondrial DNA (mtDNA) content related to actin gene was measured using Real-time PCR as described previously. The data of TA was not significantly different from control group (data not shown).

Statistics analysis: Data are shown as the mean values ± S.E. Results of two groups were analyzed using "unpaired" Student’s t tests. P < 0.05 was taken to be statistically significant. (*, P < 0.05, **, P < 0.01)

RESULTS
Muscle Weight: Table 1 shows that the change of weight of TA and SOL.

Mitochondria Number: Mitochondria number in TA was increased but not significantly. Whereas, mitochondria number in SOL at days 7, 14, and 21 day, there were significantly differences between two groups.

DISCUSSION
Considering the result of muscle weight and gene expression analysis, the muscle atrophy might occur at early phase (days 7 and 14), and the atrophy of SOL was recovered at late phase (days 21) in CO$_2$ group after femoral shaft fracture.

In skeletal muscle, there are two major classifications of fiber type: Type I and II. Moreover Type II can be classified as IIA, IID and IIB. These fibers have individually different characteristics of production of ATP, mitochondria number, and capillary density. IIA and IID fibers have a higher percentage of these substances than IIB.

Considering the result of mitochondria number, the muscle fiber switching from IIB to IIA might occur after days 7.

Transcutaneous CO$_2$ application could alleviate muscle atrophy after fracture in rat.

SIGNIFICANCE
In the clinical situation, we sometimes meet with muscle atrophy after fracture and post operative situation. Transcutaneous CO$_2$ application will be helpful to recover from muscle damages and muscle atrophy after injury.

REFERENCES

Table 1: In TA and SOL at day 14 and day 21, there were significantly differences between two groups.

<table>
<thead>
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<th></th>
<th>Control</th>
<th>CO$_2$</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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