

# Ultrasonic Evaluation of Muscle Recovery Following Free Functioning Gracilis Transfer, A Preliminary Study

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## Research

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# Abstract

**Background:** Ultrasonic measurement has not been utilized to assess functional recovery of transplanted muscle. This study aimed to investigate the feasibility of B-ultrasound measurement for evaluation of muscle recovery following free functioning gracilis transfer.

**Methods:** From January 2009 to January 2014, 35 patients receiving free functioning gracilis transfer due to total brachial plexus injury were enrolled. B-ultrasound was utilized to measure the cross-sectional area (CSA) of transplanted gracilis muscle at rest and contraction state. The ratio of pre- to post-transplant CSA value at rest state was defined as muscle bulk ratio (MBR). The ratio of CSA value at the contraction state to that at rest state was defined as contraction ratio (CR).

**Results:** Patients with muscle strength  $M \geq 4$  had significantly higher CR1 (post-transplant), CR2 (pre-transplant), and range of motion (ROM, joint mobility) than those with muscle strength  $M < 4$ . The  $CR1 > CR2$  group had significantly higher CR1, muscle strength, and ROM than the  $CR1 \leq CR2$  group. The  $MBR > 1$  group had significantly higher muscle strength than the  $MBR \leq 1$  group. CR1 value was highly correlated with muscle strength and with ROM. CR2 value was moderately correlated with muscle strength and ROM. Multivariate linear regression analysis showed that higher CR1/CR2 value was associated with higher muscle strength and higher joint mobility. The  $CR1 > CR2$  group had better muscle strength and ROM than the  $CR1 \leq CR2$  groups.

**Conclusion:** B-ultrasound measurement can quantitatively reflect muscle strength following gracilis transfer, and CR but not MBR value could be a potential indicator for functional recovery of the transplanted gracilis muscle.

**Level of Evidence:** Prognostic studies, Level II.

## Background

Functional free gracilis muscle transfer is a surgical procedure for limb motor function in patients with Brachial plexus avulsion, brachial plexus nerve injuries, traumatic muscle loss, and tumor resection.[1–7] It transplants gracilis muscles with vascular nerve pedicles to the upper limbs, suturing the affected blood vessels and motor nerves to replace the function of lost muscles in the affected area.[8] The reinnervation after gracilis muscle transfer is a slow process, and according to the recovery status, auxiliary surgery (such as fusion of wrist joint) is usually required to promote functional recovery.[9, 10] Therefore, timely assessing functional recovery of transplanted muscle can provide a reference for subsequent decision-making.

Currently, clinically available methods for assessing functional recovery of transplanted muscle include electromyography,[11] manual muscle test (MMT),[12] and magnetic resonance imaging (MRI).[13] However, all these evaluation methods have some defects. EMG results are not necessarily consistent with muscle strength recovery of the transplanted gracilis muscle. MMT is easy to conduct but subjective,

and muscle strength M4 cannot be quantitatively assessed. MRI cannot dynamically assess muscle contraction function, and it is expensive.

B-ultrasound is non-invasive, convenient, and cheap, which can reflect muscle strength by echo density, muscle thickness, cross-sectional area, and intermuscular volume.[14–18] Compared with the static observation of MRI, B-ultrasound can observe the dynamic contraction of muscle. Ultrasonic measurement can easily obtain morphological data of muscle, such as muscle thickness and cross-sectional area. The cross-sectional area measured by B-ultrasound has been used to investigate the effect of resistance training on muscle strength.[15, 19] Since muscle morphological parameters are closely related to muscle function, muscle strength and function can be reflected by measuring morphological parameters in ultrasonic measurement.[19] Although the correlation between morphologic parameters by ultrasonic measurement and muscle strength has been demonstrated.[15, 20, 21] However, there is no study on the application of B-ultrasound in the evaluation of functional recovery of transplanted muscle following free functioning gracilis transfer. In this study, therefore, we aimed to investigate the feasibility of B-ultrasound measurement for evaluation of muscle recovery following free functioning gracilis transfer.

## **Methods**

### **Participants**

From January 2009 to January 2014, 35 patients receiving free functioning gracilis transfer due to total brachial plexus injury at our hospital were enrolled. All patients received free functioning gracilis transfer for the first time to reconstruct the functions of elbow flexion – finger/thumb extension, and the ipsilateral accessory nerve was used as a donor motor nerve. The reconstruction of the transplanted gracilis muscle was located from the lateral side of the clavicle to the dorsal metacarpal extensor muscle and the tendon of long extensor muscle of thumb at the dorsal side of the distal forearm. This study was approved by the institutional review board of our hospital. Written informed consent was obtained from each patient.

### **Muscle strength measurement and range of motion (ROM)**

The muscle strength was assessed by manual muscle strength test and the ROM of elbow flexion was determined by a protractor tool. All assessment was performed by a trained physician who was not involved in the ultrasonic measurement. To avoid observation bias, the definition of M4 strength was modified to “ muscle can resist at least the examiner’s one finger or at least 1 kg in weight” according to Lin et al.’s study [22].

### **Ultrasonic measurement and outcome analysis**

All B-ultrasound examinations are performed using the Venue 40 Ultrasound (GE Healthcare, USA) and the cross-sectional area (CSA) of the with gracilis muscle were obtained based on the ultrasound images with the Scion Image software (National Institutes of Health, Bethesda MD). All the process was performed by the same physician and can be completed within 10 min. Before and after transplantation, the ultrasound examinations and corresponding CSA of transplanted gracilis muscle was measured at rest and at contraction state, respectively. Each ultrasound examination was continuously repeated five times and the corresponding CSA value was averaged.

Before transplantation, the CSA of the gracilis muscle at rest state was measured at the neutral position of the hip joint, while and the CSA of the gracilis muscle at the contraction state was determined at 30°abduction of the hip joint.

For CSA measurement after transplantation, the patient was placed in the supine position, and the upper extremity was straightened. The midpoint of the transplanted gracilis muscle was determined by B-ultrasound scan, and the plane was used to measure the CSA of the gracilis muscle at rest state. The CSA of the gracilis muscle at the contraction state was determined at 20 ° abduction of shoulder, wrist neutral position, 60 ° flexion of elbow, which was the posture used to restore the original length of the transplanted muscle during surgery. Restoring the original length of the transplanted muscle in this position will not cause the elbow joint straightening obstacle, but also can achieve the best elbow flexion-finger extension effect. Therefore, patients received B-ultrasound examination in the same posture to make the pre-transplant and post- transplant results comparable.

## **Muscle bulk ratio (MBR) and contraction ratio (CR)**

The ratio of pre- to post-transplant CSA value of gracilis muscle at rest state was defined as MBR. The ratio of CSA value at the contraction state to that at rest state was defined as contraction ratio (CR). The CR values of gracilis muscle before and after transplantation were defined as CR-2 and CR-1, respectively (Fig. 1). The patients were divided into following dichotomous groups for subgroup analysis of muscle strength or ROM: the MBR > 1 vs. MBR < 1, CR-1 > CR-2 group vs. CR-1 < CR-2 group, muscle strength  $\geq$  M4 vs. muscle strength < M4 groups.

## **Statistical analysis**

Continuous variables were presented as mean  $\pm$  SD (standard deviation) while categorical data were indicated by number and percentage (%). Student's independent t-test was used to compare the differences between groups. If normality of continuous variables was not assumed, non-parametric analysis Mann-Whitney U test would be used instead. Chi-square test and Fisher's exact test (if any expected value lower than 5 was observed) were used for categorical data. Pearson's correlation coefficient was used to report the correlation among continuous variables. Univariate and multivariate linear regression models were used to investigate the associations between CR/MBR and muscle strength/range of motion (ROM). A p-value lower than 0.05 would be recognized as reaching significance

of each test. All analyses were performed using IBM SPSS Version 20 (SPSS Statistics V20, IBM Corporation, Somers, New York).

## Results

### Patient's clinical characteristics

A total of 35 patients (32 males and 3 females, mean age =  $30.40 \pm 8.40$  years) undergoing free functioning gracilis transfer were included. The mean follow-up period of all patient's was  $19.11 \pm 6.51$  (range: 8 to 32) months after transplantation. Table 1 demonstrated demographic and clinical characteristics, including muscle strength (M), CR1 (post-transplant), CR2 (pre-transplant), MBR and ROM. The detailed ultrasonic measurement outcomes and muscle strength of all 35 patients were summarized in Supplementary Table S1.

Table 1  
Patient's clinical characteristics

Parameters	Mean ± SD / N (%)
Gender	
Male	32 (91.43)
Female	3 (8.57)
Age, year	30.40 ± 8.40
Follow-up period, month	19.11 ± 6.51
Muscle strength (M)	3.26 ± 0.89
Muscle strength group	
M < 4	17 (48.57)
M ≤ 4	18 (51.43)
CR1	1.23 ± 0.15
CR2	1.22 ± 0.13
Relation between CR1 and CR2	
CR1 > CR2	20 (57.14)
CR1 ≤ CR2	15 (42.86)
MBR	1.00 ± 0.14
MBR group	
> 1	18 (51.43)
≤ 1	17 (48.57)
ROM	73.66 ± 27.39
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;	
CR1 was the post-transplant CR value, while CR2 was the pre-transplant CR value.	

## Subgroup analysis stratified by muscle strength, relative CR, and MBR

The ultrasonic measurement outcomes and muscle strength were compared by the following dichotomous groups, including muscle strength (M < 4 vs. M ≥ 4), CR value order (CR1 > CR2 vs. CR1 ≤

CR2), and MBR (MBR > 1 vs. MBR ≤ 1). It was found that the M ≥ 4 group had significantly higher CR1, CR2, and ROM values than the M < 4 group (all P < 0.001, Table 2). The CR1 > CR2 group had significantly higher CR1, muscle strength, and ROM than the CR1 ≤ CR2 group (all P < 0.01, Table 3). The MBR > 1 group had significantly higher muscle strength than the MBR ≤ 1 group (P = 0.038, Table 4).

Table 2  
Comparisons between muscle strength groups

Parameters	M < 4	M ≥ 4	P
CR1	1.10 ± 0.06	1.35 ± 0.10	< 0.001
Muscle strength	2.47 ± 0.62	4.00 ± 0.00	< 0.001
ROM	52.12 ± 22.22	94.00 ± 11.54	< 0.001
CR2	1.15 ± 0.09	1.30 ± 0.12	< 0.001
MBR	1.02 ± 0.18	0.99 ± 0.09	0.576
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;			
CR1 was the post-transplant CR value, while CR2 was the pre-transplant CR value.			

Table 3  
Comparisons between comparative relation of CR groups

Parameters	CR1 > CR2	CR1 ≤ CR2	P
CR1	1.29 ± 0.15	1.14 ± 0.11	0.002
Muscle strength	3.60 ± 0.68	2.80 ± 0.94	0.006
ROM	85.20 ± 22.40	58.27 ± 26.39	0.003
CR2	1.21 ± 0.12	1.24 ± 0.15	0.483
MBR	0.98 ± 0.11	1.04 ± 0.17	0.237
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;			
CR1 was the post-transplant CR value, while CR2 was the pre-transplant CR value.			

Table 4  
Comparisons between MBR groups

Parameters	MBR > 1	MBR ≤ 1	P
CR1	1.24 ± 0.14	1.21 ± 0.17	0.660
Muscle strength	3.56 ± 0.51	2.94 ± 1.09	0.038
ROM	78.67 ± 16.98	68.35 ± 35.06	0.272
CR2	1.24 ± 0.11	1.21 ± 0.15	0.500
MBR	1.11 ± 0.11	0.89 ± 0.05	< 0.001
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;			
CR1 was the post-transplant CR value, while CR2 was the pre-transplant CR value.			

## Associations of CR/MBR to M/ROM

To further evaluate the potential of ultrasonic outcomes as the indicator for muscle strength, correlation analysis between CR/MBR and muscle strength/ROM was performed. As shown in Table 5, CR1 had the highest correlation coefficient with muscle strength (0.808) and ROM (0.847) (both  $P < 0.01$ ); CR2 had a medium correlation coefficient with M (0.491) and ROM (0.556) (both  $P < 0.01$ ). However, MBR had no significant correlation with muscle strength and ROM (both  $P > 0.05$ ). The coefficient between M and ROM was 0.903 ( $P < 0.001$ ).

Table 5  
Correlation coefficient results

Parameters	CR1	CR2	MBR
Muscle strength	0.808**	0.491**	0.204
ROM	0.847**	0.556**	0.017
**, $P < 0.001$ .			
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;			
CR1 was the post-transplant CR value, while CR2 was the pre-transplant CR value.			

The associations between CR/MBR and M/ROM were further evaluated by univariate and multivariate linear regression analysis. Multivariate linear regression analysis adjusted for patient's age and gender showed that higher CR1/CR2 value suggested higher muscle strength and ROM value (all  $P < 0.01$ , Table 6). Meanwhile, the CR1 > CR2 group had better muscle strength and ROM than the CR1 ≤ CR2

groups (all  $P < 0.01$ , Table 6). However, there were no significant results in the regression models of MBR (all  $P > 0.05$ ).

Table 6  
Univariate and multivariate linear regression results

Parameters	Univariate		Multivariate <sup>1</sup>	
	B <sup>2</sup>	P	B	P
Muscle strength				
CR1	4.70 (3.49–5.91)	< 0.001	4.61 (3.37–5.84)	< 0.001
CR2	3.35 (1.24–5.45)	0.003	3.47 (1.35–5.59)	0.002
Relation between CR1 and CR2				
CR1 > CR2	ref.	-	ref.	-
CR1 ≤ CR2	-0.80 (-1.36–0.24)	0.006	-0.80 (-1.39–0.21)	0.010
MBR	1.30 (-0.90-3.51)	0.239	1.50 (-0.75-3.74)	0.183
ROM				
CR1	152.31 (118.49-186.13)	< 0.001	150.44 (115.59-185.29)	< 0.001
CR2	117.16 (55.06-179.27)	< 0.001	122.02 (59.24-184.79)	< 0.001
Relation between CR1 and CR2				
CR1 > CR2	ref.	-	ref.	-
CR1 ≤ CR2	-26.93 (-43.73–10.14)	0.003	-27.34 (-45.41–9.27)	0.004
MBR	3.26 (-66.37-72.90)	0.925	7.87 (-64.01-79.74)	0.825
<sup>1</sup> , multivariate results was adjusted with patient's age and gender.				
<sup>2</sup> , B stands for regression coefficient.				
CR, contraction ratio; MBR, muscle bulk ratio; ROM, range of motion;				
CR1 was the post-transplant CR value, while CR2 was the pre- transplant CR value.				

## Discussion

In this study, we investigated the feasibility of B-ultrasound measurement for evaluation of muscle recovery following free functioning gracilis transfer. The results showed that The  $M \geq 4$  group had significantly higher CR1, CR2, and ROM value (joint mobility) as compared with the  $M < 4$  group. The  $CR1 > CR2$  group had significantly higher CR1, muscle strength, and ROM than the  $CR1 \leq CR2$  group. The  $MBR > 1$  group had significantly higher muscle strength than the  $MBR \leq 1$  group. CR1 value was highly correlated with muscle strength ( $r = 0.808$ ) and ROM ( $r = 0.847$ ), while CR2 value was moderately correlated with muscle strength ( $r = 0.491$ ) and ROM ( $r = 0.556$ ). Multivariate linear regression analysis showed that higher CR1/CR2 value was associated with higher muscle strength and higher joint mobility. Meanwhile, the  $CR1 > CR2$  group had better muscle strength and ROM than the  $CR1 \leq CR2$  groups (all  $P < 0.01$ ). Taken together, these results suggested that B-ultrasound measurement can quantitatively reflect muscle function following gracilis transfer, and CR but not MBR value could be a potential indicator for muscle function recovery.

Currently, ultrasonic parameters which could be used as the indicator for muscle contraction include echo intensity (EI),[20] muscle thickness,[23] muscle fiber pennation,[24] measures of muscle architecture.[18] EI reflects muscle function by the density of high echo signals in the muscle but is susceptible to be affected by the connective tissue between the subcutaneous and muscle bundles. A study on the changes in muscle strength by Jacobs et al. has confirmed that EI is not an optimal indicator of muscle strength.[20] In addition, the transplanted gracilis muscle undergoes denervation and fibrosis, therefore EI is not suitable for function evaluation following free functioning gracilis transfer. Muscle fiber pennation reflects the muscle contraction by the angle between the direction of muscle fibers and the long axis of the muscle.[25] Gracilis muscle is a non-feathery muscle, and could not be measured by muscle fiber pennation. The transplanted muscle is close to the skins, and the ultrasonic test is susceptible to be affected by probe-induced pressure, hence, muscle thickness measurement is also not applicable for muscle transplantation.

The gracilis muscle is non-feathery muscle, and its muscle fibers are arranged in the same direction as the tendon. During the muscle contraction, all the muscle fibers slide in parallel. Therefore, the histological cross-sectional area of the muscle fibers of the gracilis muscle is substantially the same as the gross anatomical cross-sectional area.[18] Therefore, B-ultrasound can be used to measure CSA, and the dynamic contraction of muscles can be reflected by CSA changes (ie, CR) at rest and contraction. The transplanted gracilis muscle undergo denervation and nerve re-innervation, and the muscle volume changes during this process. The MBR and CR are standardized indicators calculated based on CSA, which can eliminate the impact of muscle volume change during denervation and nerve re-innervation. [26] Hence, we chose CR and MBR as the indicators to explore the recovery of gracilis muscle functional after free functioning gracilis transfer.

Our results showed a high correlation between CR value and muscle function indexes, indicating that CR can be used to dynamically evaluate the recovery of muscle function after transplantation. In addition, muscle strength and ROM were significantly higher in the  $CR1 > CR2$  group than in the  $CR1 \leq CR2$  group, suggesting that when the muscle CR increases after transplantation, the patient has better recovery of

muscle strength and joint mobility. We observed an increase in the CR value of the gracilis muscle after transplantation. One of the possible reasons is as follows: Since the motion range of elbow bowing is larger than hip adduction, the muscle fibers of transplanted gracilis muscle need to be parallel sliding for a longer distance in the elbow bowing. Therefore, the CR value was elevated after transplantation. Our results showed that the mean CR1 values of the muscle strength  $\geq$  M4 group and the CR1 > CR2 group were  $1.35 \pm 0.10$  and  $1.29 \pm 0.15$ , respectively. Based on these results, we propose that CR1 of 1.3 might be an important reference value for transplanted muscle recovery. When the CR value of transplanted muscle reaches 1.3, the muscle might have satisfactory recovery. However, this reference value should be further validated in a large trial.

In this study, we also evaluated MBR, which reflects muscle volume change, ie atrophy (MBR < 1) or hypertrophy (MBR > 1). We found that patients who had no atrophy with gracilis muscle (MBR > 1) showed no significant advantage in muscle strength and joint mobility. Subgroup analysis of muscle strength also showed that MBR value was not significantly different between two muscle strength groups. Correlation analysis also revealed that there was no correlation between MBR and muscle strength/joint mobility. All these results suggested that MBR is not suitable as an indicator for evaluating muscle recovery following free functioning gracilis transfer.

Our preliminary findings demonstrated the feasibility of utilizing B ultrasound to evaluate functional recovery after gracilis transfer. Early muscle contraction changes may not be easily detected by physical examination. B-ultrasound examination can dynamically detect the progress of muscle contraction recovery, as well as the tendon-gliding function. When a lower postoperative CR value is found in early postoperative period, the patients can seek the help of rehabilitation doctors for physical therapy to promote nerve reinnervation. If in the late postoperative period (e.g. at 1 year after surgery, there is no progress in muscle strength for 3 consecutive months), a second femoral muscle transplantation could be performed to rebuild the flexor. Even though this was a prospective study, however, the sample size was relatively small. However, In the future, a large trial should be conducted to validate the findings of this study and define the reference values for functional recovery assessment.

## Conclusions

In summary, our findings demonstrated that B-ultrasound measurement can quantitatively reflect muscle function following gracilis transfer, and CR but not MBR could be a potential indicator for muscle function recovery. The CR value of transplanted muscle was highly correlated with the muscle function, which can be used to dynamically assess muscle recovery after muscle transplantation. Elevated CR value of gracilis muscle after transplantation suggested a better prognosis.

## Abbreviations

MMT: manual muscle test; MRI: magnetic resonance imaging; ROM: range of motion; CSA: cross-sectional area; MBR: Muscle bulk ratio; CR: contraction ratio

# Declarations

## Ethics approval and consent to participate

This study was approved by the institutional review board of the First Affiliated Hospital of Sun Yat-Sen University (No. [2014]161). Written informed consent was obtained from each patient.

## Consent for publication

Written informed consent was obtained from each patient for the publication of this study.

## Availability of data and materials

All the data and materials have been presented in the main paper.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

We declare that all the listed authors have participated actively in the study and all meet the requirements of the authorship. Drs. Liqiang Gu and Jia Zheng designed the study and wrote the protocol, Drs. Yi Hou and Jiantao Yang acquired and interpreted the data, Dr. Bengang Qin managed the literature searches and analyses. Dr. Yi Hou wrote the first draft of the manuscript. All authors approved the final version of manuscript.

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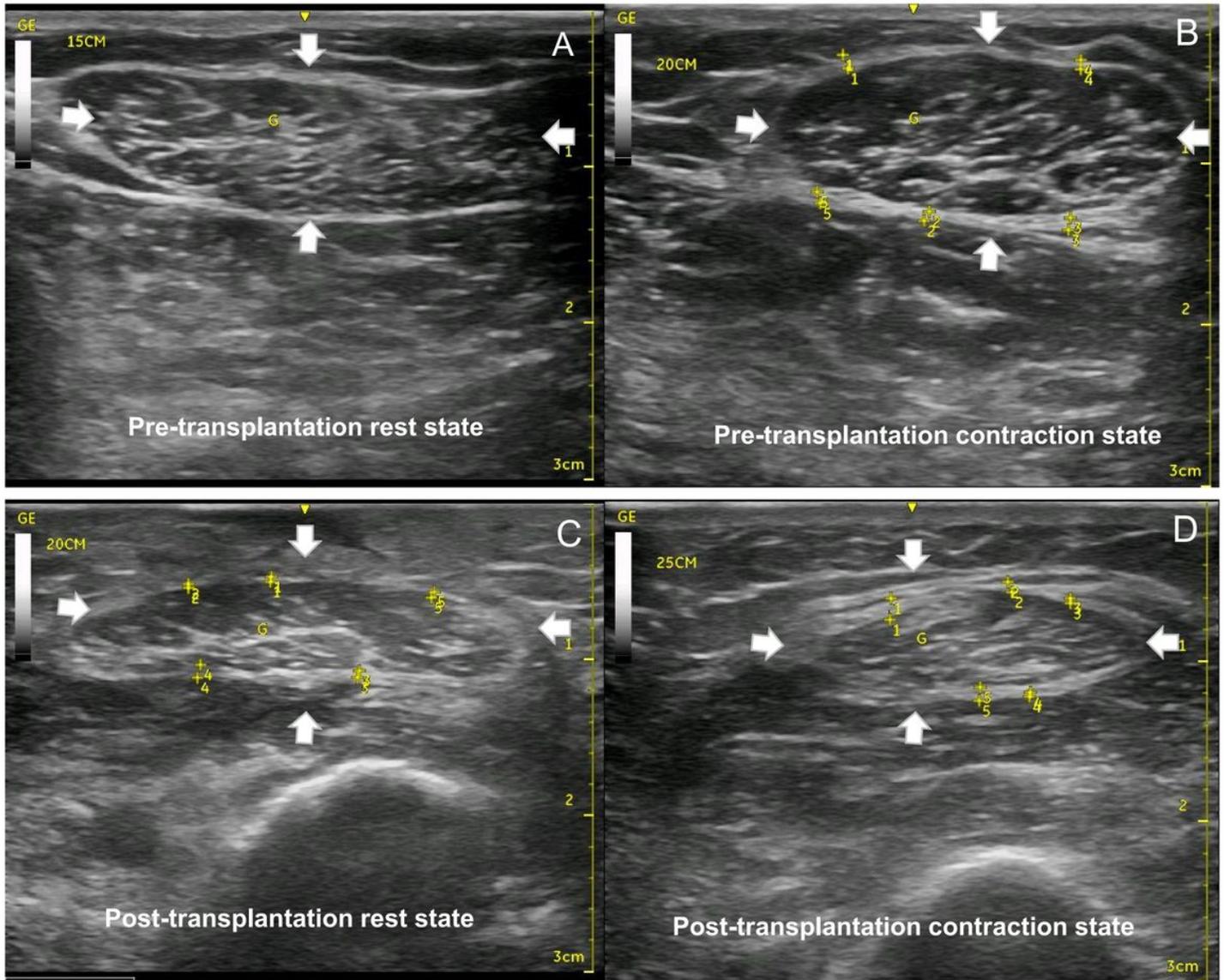
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## Figures



**Figure 1**

Ultrasound image of cross-sectional area of the gracilis muscle. The white arrows indicated muscle cross-section area (CSA). (A) Pre-transplantation rest state; (B) Pre-transplantation contraction state; (C) post-transplantation rest state; (D) post-transplantation contraction state. The ratio of pre- to post-transplant CSA value of gracilis muscle at rest state was defined as muscle bulk ratio (MBR). The ratio of CSA value at the contraction state to that at rest state was defined as contraction ratio (CR). The CR values of gracilis muscle before and after transplantation were defined as CR-2 and CR-1, respectively.  $MBR = CSA \text{ in (C) } / CSA \text{ in (A)}$ ;  $CR1 = CSA \text{ in (D) } / CSA \text{ in (C)}$ ;  $CR2 = CSA \text{ in (B) } / CSA \text{ in (A)}$ .

## Supplementary Files

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