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Title
Reorganisation of the primary motor cortex following lower-limb amputation for vascular disease: A pre-post amputation comparison

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Abbreviated Title: Cortical reorganisation following amputation

Keywords: Transcranial magnetic stimulation; amputation; motor reorganisation; motor cortex; human.
Abstract

Purpose: This study compared bilateral corticomotor and intracortical excitability of the primary motor cortex (M1), pre and post unilateral transtibial amputation. Method: Three males aged 45, 55 and 48 years respectively who were scheduled for elective amputation and thirteen (10 male, 3 female) healthy control participants aged 58.9 (SD 9.8) were recruited. Transcranial magnetic stimulation assessed corticomotor and intracortical excitability of M1 bilaterally. Neurophysiological assessments were performed 10 (SD 7) days prior to surgery and again at 10 (SD 3) days following surgery. Data were analysed descriptively and objectively compared to 95% confidence intervals from control data. Results: Prior to amputation, all three patients demonstrated stronger short-latency intracortical inhibition evoked from M1 ipsilateral to the affected limb and reduced long-latency intracortical inhibition evoked from M1 contralateral to the affected limb compared to control subjects. Following amputation, short-latency intracortical inhibition was reduced in both M1s and long-latency intracortical inhibition was reduced for the ipsilateral M1. Single-pulse motor evoked potential amplitude and motor thresholds were similar pre-to-post amputation. Conclusions: Modulation of intracortical excitability shortly following amputation indicates that the cortical environment may be optimized for reorganisation in the acute post-amputation period which might be significant for learning to support prosthetic mobility.
Introduction

Animal and human studies have established amputation is associated with extensive reorganisation of the primary motor cortex (M1) [1-4]. In long-term amputees reorganisation primarily occurs at the level of the cortex [5,6], with expansion of adjacent cortical representations within M1 contralateral to the amputated limb (M1CON) [6-8]. In addition, there is increased corticomotor excitability [5,7,9] and activation of a larger percentage of the motoneuron pool [5-7]. Reorganisation of M1CON is likely mediated by intracortical inhibitory GABAergic interneurons [5], while in upper-limb amputees an increase in activity of facilitatory glutamatergic interneurons has been demonstrated [10]. Interestingly, unilateral amputation is also linked with reorganisation of the ipsilateral M1 (M1IPSI). Lateral displacement of the ipsilateral motor map was reported in long-term lower-limb amputees [11], and modulation of inhibitory GABAergic interneurons and cortical excitability was observed bilaterally in post-acute lower-limb amputees completing prosthetic rehabilitation [12,13]. Since normal cortical control of an individual lower-limb involves both hemispheres [14-16], reorganisation of M1IPSI alongside that of M1CON post-amputation is unsurprising. However, increased demand of the non-amputated limb [11,12], or interhemispheric projections from the reorganizing M1CON may also facilitate reorganisation of M1IPSI [17]. Significantly from a rehabilitation perspective, there is some indication that these patterns of bilateral cortical reorganisation in long-term amputees may be associated with prosthetic mobility, with increased cortical excitability of ipsilateral projections from the ipsilateral M1 to the amputated limb detrimental to gait function [12,13,16]. The neurophysiology of bilateral cortical reorganisation following amputation warrants further investigation.

While current research has predominantly focused on long-term amputees, there is little understanding of cortical reorganisation during the acute phase which may impact on
rehabilitation. A greater understanding of cortical neuronal function after amputation may identify critical periods where the brain is more amenable to reorganisation, assisting identification of effective early rehabilitation interventions. Interestingly, literature indicates prosthetic rehabilitation services begin anywhere from 9 days to 1 month post-surgery [18], with those suitable for prosthetic rehabilitation not beginning mobility training until 1 to 3 months post-surgery [18-21]. It may be that the acute post-amputation period, prior to beginning prosthetic mobility, is a critical window for functionally adaptive cortical reorganisation and may currently be underutilized. Furthermore, it is unclear if patterns of reorganisation which are associated with poor prosthetic function [12,13,16] manifest in the acute pre-prosthetic, post-amputation period, and this may be a critical period to drive adaptive cortical reorganisation and improve prosthetic gait. Characterizing acute post-amputation neurophysiology may address these important questions and provide evidence to support research into augmentation of neuroplasticity during this critical period, with significant implications for early rehabilitation.

While cortical reorganisation in the acute post-amputation period may be associated with recovery, it is unclear if reorganisation prior to amputation is associated with post-amputation function. Common pathologies leading to lower-limb amputation, such as peripheral vascular disease and type II diabetes mellitus [18,22], might also drive cortical reorganisation prior to amputation. It is feasible to suggest corticomotor excitability is influenced by pathology, as previous studies suggest prolonged central motor conduction time in diabetics [23,24]. This reorganisation is likely driven by peripheral neuropathy impairing afferent input or central neuropathy impairing neural conduction in the central nervous system [23]. Animal studies indicate that central mechanisms driving cortical reorganisation in diabetes may be impaired maintenance of NMDA dependent long term potentiation [25]. The only report of cortical
reorganisation pre-to-post amputation was in a single case following traumatic transradial amputation where common pathologies leading to amputation are not relevant [26]. Similar studies in lower-limb amputations have not been conducted due to the difficulty in recruitment of appropriate participants prior to elective amputations and the challenges associated with stimulating lower-limb cortical representations which are less accessible given their medial location. Given the prevalence of diabetic and vascular amputations, an investigation of this population may address relevant questions regarding pre-amputation and acute post-amputation neurophysiology. The purpose of this preliminary study was to compare corticomotor and intracortical excitability of M1 between impending amputees and healthy controls to understand neurophysiology prior to amputation. Second, we sought to understand the acute neurophysiology associated with lower-limb amputation by investigating corticomotor and intracortical excitability pre-to-post surgery. The hypothesis was that corticomotor excitability would increase and intracortical GABAergic inhibition and glutamatergic facilitation would decrease in both motor cortices after amputation. Modulation of corticomotor and intracortical excitability would indicate a neural environment supporting reorganisation.

Methods and Materials

Participants

Three male adults aged 49.3 (SD 5.1) years with diabetes mellitus type II and clinically diagnosed microvascular and macrovascular disease that were scheduled for elective unilateral transtibial amputations were recruited from the vascular wards. Patients scheduled for amputation were recruited from a sample of convenience over a 12 month period between 2012-2013. Amputation surgery occurred for patient 1 in August 2012, patient 2 in April 2013 and patient 3 in July 2013. Thirteen healthy adult control participants (10 male, 3
female) of similar age (58.9 (SD 9.8) years) were recruited for comparison from a sample of
convenience using a database of volunteers. Healthy adult control participants were
independently mobile, with no neurological or musculoskeletal disorders limiting functional
capacity or independence. Furthermore, healthy adults were not taking any nutritional
supplements and had not undergone any prior medical or surgical procedures which may alter
neurophysiological recordings. Limb dominance was assessed with the Edinburgh
Handedness Inventory [27]. In control participants, the non-dominant limb was modelled as
the amputated limb. Patients and healthy controls with contraindications for transcranial
magnetic stimulation (TMS) and medications known to alter central nervous system
excitability were excluded [28]. Ethical approval was provided by the Southern Adelaide
Clinical Human Research ethics committee and all participants provided informed consent.

Data Collection

Demographic and clinical characteristics of the three patients scheduled for elective
amputation were collected from the clinical case notes and reported as documented by the
medical care team. Frailty prior to amputation was assessed using the Clinical Frailty Scale
[29]. Following lower-limb amputation, phantom pain was assessed with the pain component
of the Prosthetic Evaluation Questionnaire [30]. Stump length was measured from the medial
knee joint line to the distal end of the stump. Neurophysiological assessments were conducted
prior to and following amputation, once medically stable. For healthy adult control subjects,
neurophysiological assessments were conducted upon recruitment. For both amputee patients
and healthy control subjects, neurophysiological experimental procedures were performed
with participants seated comfortably with hip and knee joints flexed to 90°. A seated knee-
extension task was performed to unilaterally pre-activate the rectus femoris (RF) muscle prior
to each TMS pulse in order to evoke a motor evoked potential (MEP) of greater than 100µV
amplitude when stimulating lower-limb cortical representations [13,16]. Consistent muscle activation at 10-15% maximal voluntary contraction was monitored with visual feedback of raw electromyography (EMG) signal from the RF. TMS pulses were triggered during muscle contractions using Signal software (Signal v5.09, Cambridge Electronic Design) at a frequency of 0.2Hz±10%.

**Electromyography**

Surface EMG was recorded bilaterally from RF using 10mm-diameter Ag/AgCl electrodes (Ambu, Denmark). Electrodes were placed 2cm apart over the muscle bellies, with the distal electrode positioned 12cm proximal to the superior pole of the patella. A 20mm-diameter reference Ag/AgCl electrode was placed over the patella (3M Health Care, St. Paul, MN, USA). Electromyography signals were sampled at 2000Hz (CED 1401; Cambridge Electronic Design), amplified (CED 1902; Cambridge Electronic Design), band-pass filtered (20-1000Hz) and stored for offline analysis (Signal v5.09, Cambridge Electronic Design).

**Transcranial Magnetic Stimulation**

Identical neurophysiological assessments were performed for both amputee patients and healthy adults control subjects. Single-pulse TMS was delivered using a Magstim 200 stimulator, and paired-pulse TMS was delivered using two stimulators connected to a BiStim² unit (Magstim Company, Dyfed, UK). A flat 70mm wing diameter, figure-of-eight coil was held tangentially over the scalp with the handle pointing 30° posterior-medially in the transverse plane. Optimal coil orientation was determined from extensive piloting prior to the investigation. At the beginning of each assessment, the coil was initially positioned 1cm posterior, 1.5cm lateral to the vertex [12,31]. From here, the ‘hotspot’ for evoking maximal responses was determined for each session by systematically moving the coil over a 1cm grid.
Active motor threshold (AMT) was determined separately for each M1 as the minimum stimulus intensity eliciting a 100µV MEP in five of ten stimuli in the contralateral RF [32].

For single-pulse TMS, 16 MEPs were evoked at 120%AMT from each M1 in turn. The peak to peak amplitude of each MEP was measured offline and then averaged. Paired-pulse TMS was used to evoke short-latency intracortical inhibition (SICI), long-latency intracortical inhibition (LICI) and intracortical facilitation (ICF). Sixteen non-conditioned and 16 conditioned MEPs were evoked in randomized order. For paired-pulse measures, MEPs were normalized by calculating a ratio between conditioned/non-conditioned responses so that a ratio >1 represented facilitation of the test MEP and <1 represented inhibition of the test MEP. For all paired-pulse measures, the test stimulus (TS) was set to produce a half maximum MEP (50%MEPmax), to allow MEP facilitation or suppression while avoiding ceiling or floor effects. This method standardizes TS intensities across different excitability states for reliable analysis of intracortical excitability [33]. SICI was assessed using three conditioning-stimulus (CS) intensities (70%AMT, 80%AMT and 90%AMT) delivered 2ms prior to the TS [34]. LICI was assessed using a suprathreshold CS (50%MEPmax) delivered 100ms before TS [35]. ICF was assessed using a CS intensity of 80%, delivered 10ms prior to the TS [34,36].

Data Analysis

Due to the small sample size descriptive statistics were used to report demographics, clinical characteristics and neurophysiological data. An independent t-test compared age of patient and control participants. Pre-amputation and post-amputation neurophysiological data (MEP amplitude (120%AMT), SICI, LICI and ICF) were compared to the control means and 95% confidence intervals (95%CI). The 95%CI’s were used to determine if the individual amputee
data differed to the control mean [37,38]. Neurophysiological data were compared pre-to-post amputation using descriptive statistics. Test MEP amplitude for paired-pulse measures and background rmsEMG were compared descriptively.

Results

Demographics and clinical characteristics

Demographics and clinical characteristics of the three patients are provided in table 1. The mean age of the three male amputee patients was 49.3 (SD 5.1) years. The control group consisted of thirteen, right limb dominant, healthy adult control participants aged 58.9 (SD 9.8) years. There was no significant difference in age between the patients and control participants (p=0.13).

Neurophysiological assessments prior to amputation

Table 2 demonstrates differences between patient and control data using the 95% CI method. Prior to amputation, patient 1 and 3 had higher AMT for both M1s, while patient 2 had lower AMT for M1IPSI compared to controls. Patient 2 and 3 had larger MEP amplitudes compared to controls evoked from M1CON, while patient 1 had smaller MEPs evoked from M1IPSI. SICI evoked from M1CON was only stronger compared to controls for patient 1. SICI evoked from M1IPSI was stronger for all patients compared to control values. LICI was weaker for all patients when evoked from M1CON but only for patient 3 when evoked from M1IPSI. For ICF, patient 3 demonstrated greater facilitation evoked from M1CON, and patient 2 demonstrated weaker facilitation evoked from M1IPSI when compared to control values. Pre-stimulus
rmsEMG and mean test MEP amplitude for paired-pulse measures for patients and controls were similar (table 3).

Comparison of pre-amputation to post-amputation data

Neurophysiological measures pre-to-post amputation were compared descriptively for each patient (tables 2 and 4). Following amputation, AMT for both hemispheres was similar for all patients. MEP amplitudes increased in both M1s for patient 2. There was a reduction in SICI evoked from both M1s for all patients. There was a reduction in LICI evoked from M1CON following amputation for patients 1 and 3. All three patients demonstrated a reduction in LICI evoked from M1IPSI following amputation. On average ICF evoked from M1CON was variable following amputation with increased ICF for patient 1, no change for patient 2 and a reduction in ICF for patient 3. ICF evoked from M1IPSI increased for patients 2 and 3 and did not change in patient 1.

Comparison of post-amputation and control data

A difference between post-amputation and control data were objectively compared using the 95%CI method (table 2). Consistent with pre-amputation observations, there was higher AMT for both M1s for patients 1 and 3, and lower AMT for M1IPSI for patient 2. MEP amplitude evoked from both M1s was larger in patient 2 compared to controls. SICI evoked from both hemispheres was reduced in all patients. LICI evoked from M1CON was reduced compared to
controls for all patients. For M1IPSI, there was a reduction in LICI for patient 3, but LICI for
patients 1 and 2 did not differ to controls. ICF evoked from M1CON was reduced for patient 3
and stronger for patient 1, while ICF evoked from M1IPSI was stronger for patients 2 and 3,
and weaker for patient 1 compared to controls.

Discussion

This preliminary study demonstrated acute cortical reorganisation in lower-limb amputees by
comparing neurophysiological measures pre-to-post amputation surgery with control data.
Even in this preliminary study there were consistent trends in the neurophysiology for all
three patients. Since we were unable to statistically analyze our results, we utilized an
objective method, similar to previous reports, of comparing patient data to control subject
95%CIs [37,38]. Prior to amputation all patients demonstrated stronger SICI evoked from
M1IPSI and reduced LICI evoked from M1CON, compared to healthy controls. Following
amputation, there was a consistent reduction in SICI in both hemispheres, when compared to
pre-amputation measures and objectively to control values (95%CIs). Furthermore, LICI in
M1IPSI was reduced compared to pre-amputation measures. However, contrary to our
hypothesis we did not observe a consistent increase in corticomotor excitability in the acute
post-amputation period in these three patients. The current findings provide preliminary data
indicating intracortical excitability of M1 is altered bilaterally prior to, and following, lower-
limb amputation. These observations may have important clinical implications which are
discussed below.

Evidence of cortical reorganisation prior to amputation was unsurprising. The contributing
pathology, peripheral vascular disease, is associated with a prolonged period of decreased
walking, increased pain and reduced sensation pre-amputation, all of which might lead to
cortical reorganisation [17,23,24]. For the hemisphere contralateral to the diseased limb, reorganisation was marked by a reduction in LICI. However, somewhat surprising was evidence of stronger SICI evoked from the hemisphere contralateral to the sound limb. It is unclear what is driving this cortical response in the ‘unaffected’ M1. It is possible that this hemisphere is reorganizing secondary to altered biomechanics during mobility prior to amputation. Furthermore, it is likely peripheral vascular disease may have affected both limbs but to a lesser extent in the limb not scheduled for amputation and this may have increased SICI in the contralateral hemisphere. We cannot tell from the current results why corticomotor excitability was unaffected by the amputation. Since no other work has examined this group prior to amputation, the differences in cortical neurophysiology between impending amputees and controls require confirmation in a larger study.

The most noteworthy effect of amputation on the cortex was a reduction in SICI evoked from M1 bilaterally, and in LICI evoked from M1IPSI. SICI and LICI are paired-pulse TMS measures thought to represent distinct inhibitory cortical networks [39]. SICI is a measure of GABA_A receptor activity [40-42], while LICI is attributed to slow inhibitory post-synaptic potentials mediated by the GABA_B receptor [39,43,44]. Reduction in the activity of inhibitory GABAergic interneurons is an important indicator of cortical reorganisation as it facilitates strengthening of synaptic efficiency, or unmasking latent connections, to drive reorganisation of muscle representations within M1 and restore function [45]. Previous studies have demonstrated that both experimentally induced and practice dependent neuroplasticity are enhanced when intracortical inhibition is reduced as a result of ischemic nerve block [46,47]. As such, the reduction of intracortical inhibition reported in this study may indicate an increase the available network to facilitate reorganisation in the cortex. It is most likely that the reduction in SICI observed post-surgery was related to the amputation, as the pathology
contributing to reorganisation prior to amputation was associated with stronger SICI evoked from M1IPSI. Our findings of reduced intracortical inhibition post-amputation might indicate that the cortical environment is optimized for reorganisation in the acute post-amputation period. However, the link between reduced SICI and recovery remains to be proved, and the importance of this reorganisation for functional recovery requires further investigation.

However, there was no evidence that the LICI pathways were modified by amputation suggesting that GABA_B receptor mediated inhibition may not be a mechanism underpinning reorganisation at this time post-amputation. In support, our previous work demonstrated that LICI evoked from M1CON is not modulated over the sub-acute rehabilitation period and is not associated with prosthetic gait performance [13]. Although we have only reported on 3 amputees in this preliminary study, we have demonstrated for the first time that GABA_A inhibition is reduced early following lower-limb amputation, indicating the importance of GABAergic mechanisms for cortical reorganisation in the acute post-amputation period.

The mechanism contributing to bilateral M1 reorganisation in the acute post-amputation period is not directly evident from this study. However, there is some evidence that normal gait in humans is controlled by both motor cortices [14-16], and cortical drive to an individual lower-limb appears less lateralized compared to the upper-limb in healthy adults [14]. It follows logically, that unilateral amputation would influence cortical excitability of both hemispheres, given their importance for lower-limb function. A second, but equally probable explanation is that ipsilateral M1 excitability was modulated by activity in interhemispheric projections from the reorganizing contralateral M1, as previously shown following deafferentation of the upper-limb in healthy adults [17]. It is unlikely that increased use of the
non-amputated limb was responsible for increased cortical drive, as these amputees were yet to begin rehabilitation.

Findings from this study have several implications for amputee rehabilitation. First, it appears that in the acute post-amputation period the cortical environment of both hemispheres is ripe for neural reorganisation as demonstrated by reduced intracortical inhibition. The amount and type of therapy to promote adaptive reorganisation could be crucial to the success of rehabilitation during this pre-prosthetic acute period. Second, consideration should be given to rehabilitation interventions in this critical post-amputation period. Given that functional prosthetic rehabilitation may be limited in the acute post-amputation period due to post-operative pain and limitations of wound healing, alternative approaches such as motor imagery, mental practice and non-invasive brain stimulation may be appropriate to facilitate functionally adaptive reorganisation. In healthy adults, lower-limb mental practice with motor imagery was shown to induce cortical reorganisation similar to that observed with physical practice [48]. While these approaches are yet to be applied to lower-limb amputees, motor imagery has successfully relieved painful phantom sensations and promoted adaptive reorganisation in upper-limb amputees [49]. In addition, non-invasive brain stimulation techniques may be applied at this early time point to facilitate patterns of cortical reorganisation consistent with good functional recovery. Previous studies have demonstrated that non-invasive brain stimulation can be used to modulate cortical excitability and improve lower-limb function in both incomplete spinal cord injury [50] and stroke [51]. While these populations are physiologically different to amputation, they provide some level of evidence to indicate that modulation of cortical excitability can lead to functional gains. Further studies are required to determine if non-invasive brain stimulation is an appropriate intervention to improve amputee gait function. Given previous research indicating that increased cortical
excitability of the ipsilateral M1 is associated with poor gait function [16], it may be appropriate for future studies to investigate non-invasive brain stimulation to reduce cortical excitability in this hemisphere. Further studies are required to explore the clinical implications of early reorganisation of the M1 ipsilateral to the amputated limb and how this can be harnessed to optimize recovery of function. Finally, given our results indicate reduced intracortical inhibition of the ipsilateral hemisphere following amputation, suggesting this hemisphere is also amenable to reorganisation, we propose therapists should limit over-use of the non-amputated leg early after surgery. Increased activity may drive use-dependent cortical reorganisation in the ipsilateral hemisphere which may impede recovery, as greater ipsilateral cortical excitability has been previously associated with poor gait function [13,16]. However, further studies are required to investigate these suggestions prior to changing current rehabilitation approaches.

Study Limitations

While this study was unique in that it tested amputees before and after amputation, the small sample size limits interpretation of the findings. However, all three impending amputees were similar as they were scheduled for elective vascular amputation, of similar ages, mobile and non-dependent prior to amputation. Normal variability of corticomotor and intracortical excitability [52] measures over time may have influenced pre-to-post amputation measures and future studies should seek to assess participants at multiple sessions to limit the influence of time-dependent effects in the data. Furthermore, while surgical amputation techniques were similar and none of the three amputees reported phantom limb pain since surgery, the effects of deafferentation prior to surgery and post-operative pain may impact cortical reorganisation and require consideration in future investigations. Previous studies have reported that deafferentation can increase cortical excitability of proximal muscle representations [17].
However, our data does not provide any consistent evidence to indicate that cortical excitability was increased in all three impending amputees prior to surgery when compared to healthy adult control subjects. Furthermore, acute pain can increase excitability of inhibitory circuits, thereby reducing excitability of the motor cortex [53]. While our results appear to differ in that we observed a reduction in excitability of inhibitory circuits, future studies should probe the impact of both pre-operative and post-operative pain with objective measures to determine the impact of pain on neurophysiology. Finally, we acknowledge that two amputees (patient 1 and patient 3) had metatarsal amputations prior to the current transtibial amputation. While only patient 3 experienced the metatarsal amputation on the same side as the transtibial amputation, it may be that adjacent cortical representation had already undergone some level of reorganisation. Our results should be considered in light of this potential confounder.

Conclusion

In conclusion, we observed cortical reorganisation prior to amputation in three impending amputees and compared findings to a control group. It should be noted that the results presented here in three amputees are preliminary in nature, and larger studies in the future are required to confirm these findings. However, our results suggest that difference in cortical neurophysiology in amputees is likely driven by the pathology leading to amputation of the lower-limb. The most striking post-amputation cortical response was a reduction in intracortical inhibition in both hemispheres, suggesting the cortical environment is favorable for reorganisation early after amputation. Therapists should be cognizant of the impact of bilateral cortical reorganisation when designing early rehabilitation activities. Future studies seeking to improve gait function may investigate alternative rehabilitation approaches such as motor imagery, mental practice and non-invasive brain stimulation in order to utilize the
neural environment which appears optimized for reorganisation in the acute post-amputation period.
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Declaration of Interest: The authors declare no conflict of interest regarding publication of this paper.
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Legends

Table 1: Patient demographics and clinical characteristics. All three amputees were males of similar age and pre-morbid mobility and frailty.

Table 2: Neurophysiological data for individual patient’s pre and post amputation. Bold numbers indicate patient measures which were outside the 95% confidence interval from healthy control data. AMT, active motor threshold; MEP, motor evoked potential; SICI, short-latency intracortical inhibition; LICI, long-latency intracortical inhibition; ICF, intracortical facilitation; CS, conditioning stimulus intensity; M1CON, motor cortex contralateral to the amputated limb; M1IPSI, motor cortex ipsilateral to the amputated limb.

Table 3: Test MEP size (mV) of amputees (pre and post amputation) and control subjects. SICI, short-latency intracortical inhibition; ICF, intracortical facilitation; LICI, long-latency intracortical inhibition; M1CON, primary motor cortex contralateral to the amputated limb; M1IPSI, primary motor cortex contralateral to the non-amputated limb.

Table 4: A summary of descriptive findings of difference in neurophysiological measures pre-to-post amputation. ↑, increase from pre-to-post amputation; ↓, increase from pre-to-post amputation; =, no change from pre-to-post amputation.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Pre-Amputation Mobility</td>
<td>Independent, Nil Aids, &gt;50m</td>
<td>Independent, Nil Aids, &gt;50m</td>
<td>Independent, Nil Aids, &gt;50m</td>
</tr>
<tr>
<td>Clinical Frailty Scale</td>
<td>Well, with treated comorbid disease and well controlled symptoms</td>
<td>Well, with treated comorbid disease and well controlled symptoms</td>
<td>Apparently vulnerable, although not frankly dependent</td>
</tr>
<tr>
<td>Amputated Side</td>
<td>Right</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Stump Length (cm)</td>
<td>25</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>Phantom Pain</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Pre-Amputation Assessment (days prior to surgery)</td>
<td>2</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Post-Amputation Assessment (days post-surgery)</td>
<td>10</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>T2DM – 15 year diagnosis, Peripheral neuropathy - 2 year diagnosis, HT, Hyperlipidemia, GORD</td>
<td>T2DM – 18 year diagnosis, Charcot arthropathy – 3 year diagnosis</td>
<td>T2DM – 11 year diagnosis, Peripheral neuropathy – 2 year diagnosis, Diabetic retinopathy, IHD</td>
</tr>
<tr>
<td>Past Medical History</td>
<td>Left 1st and 2nd Metatarsal Amputation 2 years prior</td>
<td>Nil</td>
<td>Left 2nd Metatarsal Amputation 2 years prior</td>
</tr>
<tr>
<td>Surgical Technique</td>
<td>Posterior based flap, GA with regional block</td>
<td>Posterior based flap, GA with regional block</td>
<td>Posterior based flap, GA with regional block</td>
</tr>
<tr>
<td></td>
<td>Pre-Amputation</td>
<td>Post-Amputation</td>
<td>Control</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-----------------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>Patient 1</td>
<td>Patient 2</td>
<td>Patient 3</td>
</tr>
<tr>
<td>AMT (%MSO) M1CON</td>
<td>70</td>
<td>41</td>
<td>61</td>
</tr>
<tr>
<td>M1IPSI</td>
<td>58</td>
<td>31</td>
<td>65</td>
</tr>
<tr>
<td>MEP Amplitude (mV) M1CON</td>
<td>0.27</td>
<td>0.58</td>
<td>0.50</td>
</tr>
<tr>
<td>M1IPSI</td>
<td>0.22</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>SICI M1CON, CS 70% AMT</td>
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## Table 3

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