

# Supplementary Results for the Manuscript ‘Aberrant activity in an intact residual muscle is associated with phantom limb pain in above-knee amputees’

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## Full statistical results from the main analyses

**Table 1.** Significance of terms in the first regression model assessing EMG amplitude during movement of the feet.

	Term	Sum Sq.	Mean Sq.	Num. df	Den. df	<i>F</i>	<i>p</i>	<i>Sig.</i>
	Pre-amputation Limb Dominance	0.067	0.067	1	15	5.229	0.037	*
	Group	0.102	0.102	1	15	7.965	0.013	*
	Mov Cond	0.169	0.169	1	192	13.184	<.001	***
	Limb Status	0.401	0.401	1	192	31.244	<.001	***
	Group: Mov Cond	0.005	0.005	1	192	0.374	0.541	
	Group: Limb Status	0.406	0.406	1	192	31.666	<.001	***
	Mov Cond: Limb Status	0.006	0.006	1	192	0.459	0.499	
	Group: Mov Cond: Limb Status	0.031	0.031	1	192	2.450	0.119	

Significance of model terms was assessed using *F* tests on type III sums of squares and denominator degrees of freedom estimated using Satterthwaite method. The alpha value was set at 0.05. Mov Cond: Movement Condition.

**Table 2.** Post hoc analysis of the interaction among factors Group and Limb Status found in the first regression analysis.

	Term	Level	Contrast	Estimate	Std. Error	df	<i>t</i>	<i>p</i>	<i>Sig.</i>
Group	Control	Amp – Int	-0.001	0.022	192.0	-0.027	1.000		
		PLP	Amp – Int	0.173	0.022	192.0	7.932	<.001	***
Limb Status	Amp	Control – PLP	-0.179	0.036	22.3	-4.949	<.001	***	
		Int	Control - PLP	-0.006	0.036	22.3	-0.158	1.000	

Marginal means were computed for the 2 levels of Group and the 2 level of Limb Status (averaging over levels of Movement Condition and Limb Dominance). The Satterthwaite degrees of freedom method was used. P-values were adjusted using the Bonferroni method for 4 tests. Control: Control group. PLP: Phantom limb pain group. Amp: Amputated leg. Int: Intact leg.

**Table 3.** Significance of terms in the second regression model assessing the relationship between EMG amplitude and Phantom Limb Pain rating.

Term	Sum Sq.	Mean Sq.	Num. df	Den. df	<i>F</i>	<i>p</i>	<i>Sig.</i>
Pre-amputation Limb Dominance	0.063	0.063	1	6	4.476	0.079	
Mov Cond	0.058	0.058	1	93	4.157	0.044	*
Limb Status	0.807	0.807	1	93	57.377	<.001	***
Pain	0.114	0.114	1	6	8.082	0.029	*
Mov Cond: Limb Status	0.032	0.032	1	93	2.294	0.133	
Mov Cond: Pain	0.009	0.009	1	93	0.611	0.437	
Limb Status: Pain	0.147	0.147	1	93	10.460	0.002	**
Mov Cond: Limb Status: Pain	0.011	0.011	1	93	0.752	0.388	

Significance of model terms was assessed using *F* tests on type III sums of squares and denominator degrees of freedom estimated using Satterthwaite method. The alpha value was set at 0.05. Mov Cond: Movement Condition. Pain: Phantom Limb Pain Rating.

**Table 4.** Post hoc analysis of Limb Status x Pain interaction: Estimated marginal trends of the Pain effect grouped by Limb Status.

Term	Level	Trend	Std. Error	df	Lower CL	Upper CL
Limb Status	Amp	0.056	0.014	11.8	0.026	0.085
	Int	0.009	0.014	11.8	-0.021	0.038

Results are averaged over levels of Movement Condition and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method. Alpha value = 0.05. Confidence level = 0.95. Amp: Amputated leg. Int: Intact leg.

**Table 5.** Post hoc analysis of Limb Status x Pain interaction: Pairwise contrast of estimated marginal trends for Phantom Limb Pain Rating within levels of Limb Status.

Contrast	Estimate	Std. Error	df	<i>t</i>	<i>p</i>	<i>Sig.</i>
Amp-Int	0.047	0.015	93	3.234	0.002	**

Results are averaged over levels of Movement Condition and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method and the alpha value was set at 0.05. Amp: Amputated leg. Int: Intact leg.

### Statistical results including participant P03 (whose data was excluded from the main analyses)

For patient P03 in the PLP group, the normalized EMG amplitudes measured from the VL muscle in the amputated leg during the flexion/extension movement condition exceeded 2 (2.120). This indicated that the average EMG amplitude was greater than twice the mean amplitude obtained during the maximum voluntary contraction (MVC). Normalized EMG amplitudes for this patient also exceeded 1 (1.02) in the VL muscle of the amputated leg during the abduction/adduction movement condition. Together, these results indicate that P03 did not reach a true MVC. As a result, we could not validly normalize this subject's data for comparison with other subjects. Further analysis showed that the EMG amplitudes obtained from this patient were greater than 2.5 SD above the PLP group mean for both the flexion/extension (mean = .508, SD = .590) and abduction/adduction (mean = .325, SD = .273)

movement conditions. For these reasons, P03 was considered an outlier and their data was excluded from the main analyses presented. However, we recognize that excluding a patient with a sample size of  $n=10$  can introduce undue bias into our results. To mitigate this risk, we have included the data from P03 in the supplementary results here.

**Table 5.** *Including patient P03.* Relationship among measures of limb deficiency and TPD and phantom limb pain rating.

	Months post amputation	Prosthetic use	Pre-amputation pain	TPD intact limb	TPD amputated limb
Spearman's Rho ( $r$ )	.475	-.380	-.087	.309	.030
Sig. ( $p$ )	.165	.279	.811	.385	.385

Correlations were analyzed using Spearman's Rank Order Correlations with an alpha of 0.05.

**Table 6.** *Including patient P03.* Significance of terms in the first regression model assessing EMG amplitude during movement of the feet.

Term	Sum Sq.	Mean Sq.	Num. df	Den. df	$F$	$p$	Sig.
Pre-amputation Limb							
Dominance	0.086	0.086	1	16	2.532	0.131	
Group	0.109	0.109	1	16	3.199	0.093	
Mov Cond	0.436	0.436	1	203	12.766	<.001	***
Limb Status	1.019	1.019	1	203	29.843	<.001	***
Group: Mov Cond	0.028	0.028	1	203	0.820	0.366	
Group: Limb Status	1.028	1.028	1	203	30.103	<.001	***
Mov Cond: Limb Status	0.050	0.050	1	203	1.471	0.227	
Group: Mov Cond: Limb Status	0.107	0.107	1	203	3.137	0.078	

Significance of model terms was assessed using  $F$  tests on type III sums of squares and denominator degrees of freedom estimated using Satterthwaite method. The alpha value was set at 0.05. Mov Cond: Movement Condition.

**Table 7.** *Including patient P03.* Post hoc analysis of the interaction among factors Group and Limb Status found in the first regression analysis.

Term	Level	Contrast	Estimate	Std. Error	df	$t$	$p$	Sig.
Group	Control	Amp – Int	-0.001	0.036	203	-0.016	1.000	
	PLP	Amp – Int	0.268	0.034	203	7.955	<.001	***
Limb Status	Amp	Control – PLP	-0.294	0.092	18.5	-3.181	0.020	*
	Int	Control - PLP	-0.025	0.092	18.5	-0.267	1.000	

Marginal means were computed for the 2 levels of Group and the 2 level of Limb Status (averaging over levels of Movement Condition and Limb Dominance). The Satterthwaite degrees of freedom method

was used. P-values were adjusted using the Bonferroni method for 4 tests. Control: Control group. PLP: Phantom limb pain group. Amp: Amputated leg. Int: Intact leg.

**Table 8.** Including patient P03. Significance of terms in the second regression model assessing the relationship between EMG amplitude and Phantom Limb Pain rating.

Term	Sum Sq.	Mean Sq.	Num. df	Den. df	F	p	Sig.
Pre-amputation Limb Dominance	0.063	0.063	1	7	1.392	0.277	
Mov Cond	0.362	0.362	1	104	7.973	0.006	**
Limb Status	2.161	2.161	1	104	47.657	<.001	***
Pain	0.132	0.132	1	7	2.912	0.132	
Mov Cond: Limb Status	0.161	0.161	1	104	3.540	0.063	
Mov Cond: Pain	0.226	0.226	1	104	4.990	0.028	*
Limb Status: Pain	0.892	0.892	1	104	19.661	<.001	***
Mov Cond: Limb Status: Pain	0.111	0.111	1	104	2.447	0.121	

Significance of model terms was assessed using *F* tests on type III sums of squares and denominator degrees of freedom estimated using Satterthwaite method. The alpha value was set at 0.05. Mov Cond: Movement Condition. Pain: Phantom Limb Pain Rating.

**Table 9.** Including patient P03. Post hoc analysis of Movement Condition x Pain interaction: Estimated marginal trends of the Pain effect grouped by Movement Condition.

Term	Level	Trend	Std. Error	df	Lower CL	Upper CL
Mov Cond	F/E	0.105	0.048	8	-0.004	0.216
	Ab/Ad	0.052	0.048	8	-0.058	0.162

Results are averaged over levels of Limb Status and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method. Alpha value = 0.05. Confidence level = 0.95. Mov Cond: Movement condition. F/E: flexion/extension, Ab/Ad: abduction/adduction.

**Table 10.** Including patient P03. Post hoc analysis of Movement Condition x Pain interaction: Pairwise contrast of estimated marginal trends for Phantom Limb Pain Rating within levels of Movement Condition.

Contrast	Estimate	Std. Error	df	t	p	Sig.
F/E – Ab/Ad	0.054	0.024	104	2.234	0.027	*

Results are averaged over levels of Limb Status and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method. Alpha value = 0.05. F/E: flexion/extension, Ab/Ad: abduction/adduction.

**Table 11.** Including P03. Post hoc analysis of Limb Status x Pain interaction: Estimated marginal trends of the Pain effect grouped by Limb Status.

Term	Level	Trend	Std. Error	df	Lower CL	Upper CL
Limb Status	Amp	0.133	0.048	8	0.023	0.243
	Int	0.025	0.048	8	-0.085	0.135

Results are averaged over levels of Movement Condition and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method. Alpha value = 0.05. Confidence level = 0.95. Amp: Amputated leg. Int: Intact leg.

**Table 12.** *Including patient P03.* Post hoc analysis of Limb Status x Pain interaction: Pairwise contrast of estimated marginal trends for Phantom Limb Pain Rating within levels of Limb Status.

Contrast	Estimate	Std. Error	df	<i>t</i>	<i>p</i>	<i>Sig.</i>
Amp-Int	0.108	0.024	104	4.434	<.001	***

Results are averaged over levels of Movement Condition and Limb Dominance. Degrees of freedom were estimated using the Satterthwaite method and the alpha value was set at 0.05. Amp: Amputated leg. Int: Intact leg.