## **Mechanisms Underlying Local and Systemic Effects of Massage**

## NCT04131712

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## Statistical Design and Power

Statistical Analysis. The College of Health Sciences supports the effort of a biostatistician through the Office of Research to provide dedicated support to funded investigators. All statistical analyses will be performed using SAS© 9.4 (SAS institute, Cary NC). The sample size calculations herein have been performed with the assistance of the biostatistician. We used 4 main outcomes to determine sample size: cross sectional area (CSA), myofibrillar protein synthesis (MyoKsyn), extracellular vesicle (EV) number and mRNA levels of Rab27. These outcomes were also measured in the pilot study we performed with 6 subjects and the variance and expected differences were based on the data from the pilot study. See Table below.

% change	EV number	CSA	MyoKsyn	Rab27 mRNA
Pilot study data	0.29	6.1	36	116
Expected μ₁-μ₂	0.29	10	70	150
Standard Deviation, σ	0.294	11	76	170
Effect Size, $\delta =  \mu_1 - \mu_2 /\sigma$	0.99	0.91	0.92	0.88
n per Group	18	21	19	22

From this sample size analysis we decided to use n=22 per group, because it would give us the power to determine differences in the mRNA expression of EV biogenesis markers as well as enough power to detect changes in muscle size and protein synthesis. To determine statistically significant differences between the groups we will use a Two Way ANOVA to determine effects of ULLS and massage. Holm Sidak posthoc tests will be performed in case significance is seen with the ANOVA. We will use an unpaired t-test to test for cross over effect (UM versus UC left nonmassaged limb).