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Sample Size Considerations in Human Muscle Architecture Studies

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Abstract

INTRODUCTION—This report is a meta-analysis of the human muscle architecture literature that analyzes the number of muscles, number of subjects, and muscle fiber length coefficient of variation (CV) by body region.

METHODS—Muscle fiber length data are used to make recommendations for dissection-based architectural study sample sizes.

RESULTS—An average of 9 ± 10 (mean \pm SD) muscles and an average of 9 ± 5 subjects were reported in the 26 studies considered. Across all studies, average fiber length CV was highly variable ($18\% \pm 5\%$). This shows that sample sizes required to achieve adequate power varies by anatomical region.

DISCUSSION—Studies involving muscle architecture should consider regional variability and effect size and determine sample size accordingly.

Keywords

muscle; architecture; sample size; fiber length variation; statistical power

INTRODUCTION

Muscle architectural studies are used to describe and predict skeletal muscle structure and function. Human muscle architecture has been investigated using a variety of methods including ultrasound, magnetic resonance imaging (MRI), computed tomography (CT), histology and dissection. While imaging methods have the advantage of being non-invasive and can be performed on living humans, dissection studies provide the gold-standard method of describing muscle architecture since fiber length at a known sarcomere length can be quantified.¹ While large sample sizes are desirable in dissection studies, lack of access to cadavers, cost, and technically challenging methodology often limit the actual number of subjects reported per study. This can result in underpowered, inaccurate studies if variability is high between humans or between muscles of different anatomical areas. This can have significant clinical impact if these numbers are then used to create models that impact surgical decisions.

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The purpose of this study was to identify all of the human muscle architecture studies in the literature, determine their sample sizes, quantify fiber length coefficient of variation (CV) and then recommend adequate sample size for such studies in human muscle.

METHODS

PubMed was used to define all human muscle architecture studies published from 1968 through July 2011. Once the studies were identified, they were separated by methodology into dissection studies, ultrasound, magnetic resonance imaging (MRI), biopsy/histology, and studies utilizing a combination of these methods. We focused on those studies that utilized dissection methods to define muscle architecture, because they contain accurate measurements of muscle fiber length and sarcomere length. We analyzed the number of different muscles measured per study within a subject and the number of samples per muscle (number of subjects used). We calculated the total number of samples (number of muscles multiplied by number of subjects) and calculated the within-muscle fiber length (CV_m) for the i^{th} region according to the equation:

$$CV_i = \frac{1}{n} \cdot \sum_{m=1}^n CV_m$$

where the subscript i refers to the particular region (*e.g.*, forearm or leg; numbered 1–11), and m refers to the particular muscle within a region containing n muscles. N varies from 5 to 42 in this analysis and represents the number of times fiber length has been reported in the literature in a particular region. For example, the thigh has an n of 42, because the quadriceps and hamstring muscles are often studied. The arm (excluding shoulder and forearm which are separate regions) has only been reported 5 times. The average CV for all muscles was simply calculated as:

$$\overline{CV} = \frac{1}{11} \cdot \sum_{i=1}^{11} CV_i$$

We then used this information to determine sample sizes (number of subjects) for an independent samples t-test with $\alpha=0.05$ and power $(1-\beta)=0.80$ using the equation:²

$$n = \frac{1G \cdot (CV_i)^2}{(\ln(1 - \delta))^2}$$

where $CV_i = i^{\text{th}}$ region coefficient of variation and $\delta =$ percent expected treatment effect.

RESULTS

We examined the 163 human muscle architecture studies that used a variety of methods. Of these 163 studies, 26 used dissection methods, 63 used ultrasound, 4 used MRI, 18 used a combination of ultrasound and MRI and 5 used biopsy/histology methods. An additional 47 studies were modeling studies, descriptive anatomical studies, or diffusion tensor imaging studies. Others reported fiber length data duplicated from a previous study or did not report mean and standard deviation, thus making it impossible to calculate coefficient of variation; these studies were not included in the analysis. We distilled the 163 studies down to 26 usable dissection studies.^{3–28}

The average number of muscles measured per dissection study was 9 ± 10 (mean \pm SD) with a range of 1–28 muscles measured per study (Fig. 1A). The average number of subjects per dissection study was 9 ± 5 with a range of 1–25 subjects per study (Fig. 1B). The average total number of samples (number of muscles \times number of subjects per study) was highly variable, 71 ± 122 (range 1–567). The average fiber length coefficient of variation (\overline{CV}) by region was $18 \pm 5\%$ (range of 12–27%, Fig. 1C). Using these data in a one way ANOVA, if a treatment was expected to change fiber length by 10%, in the muscles of the thigh ($CV = 12.35\%$), a sample size of 22 subjects per group would be required for a power of 0.80 and $\alpha=0.05$ to determine a difference. Interestingly, given the same 10% treatment effect in the foot ($CV = 26.54\%$), sample size increases to 102 subjects per group since foot fiber length CV is so much greater (this is primarily due to small average fiber lengths in the foot). If the size of the treatment effect is increased to 20%, the corresponding sample sizes decrease to 5 per group in the thigh and 23 per group in the foot (Fig. 1D). Regardless of the effect size, these data demonstrate anatomical variation in fiber length CV_i and point to the need for anatomical region-specific experimental design.

DISCUSSION

Here we show that significant and systematic muscle fiber length variability exists by anatomical region, which leads to variable sample sizes required to perform adequately powered experiments. The CV is important to consider when designing studies that use muscle architecture parameters. We showed that sample sizes can vary from 5 to more than 100 depending upon the expected treatment effect and the region of the body that is being studied. Indeed, no studies have ever reported sample sizes of greater than 100 subjects, which would be considered a major undertaking.

Currently, mathematical models are often implemented using data that come from studies with few samples or models that do not properly scale to account for variability between subjects or variability between body regions and muscles. The way in which any of the architectural parameters scale with body size is unknown.²⁹ Therefore, using these models to define surgical methods, rehabilitation strategies, or motor control strategies should be considered with caution.

There has been a marked increase in the number of fiber length studies published that use ultrasound, since the seminal paper by Fukunaga and colleagues.³⁰ However, it must be emphasized that none of these studies measure sarcomere length, and thus it is not clear whether long fiber lengths reported, for example, represent short fibers containing stretched sarcomeres or whether the fibers are actually long, composed of a high number of serial sarcomeres. We advocate that studies use gold-standard dissection methodology, when possible, with relatively large sample sizes (> 10) to define human muscle architecture. Further, studies using other methods for measuring muscle architecture, such as imaging, should consider body region variability as well as any treatment effect and calculate the number of subjects needed accordingly. We use 10% and 20% treatment effect/effect size of fiber length as an example in this manuscript, but other variables of interest may have larger or smaller effect sizes. If the variable of interest in a study is a muscle architecture parameter other than fiber length, we encourage investigators to perform a similar analysis to that presented here using literature values to ensure that studies have adequate sample sizes and power.

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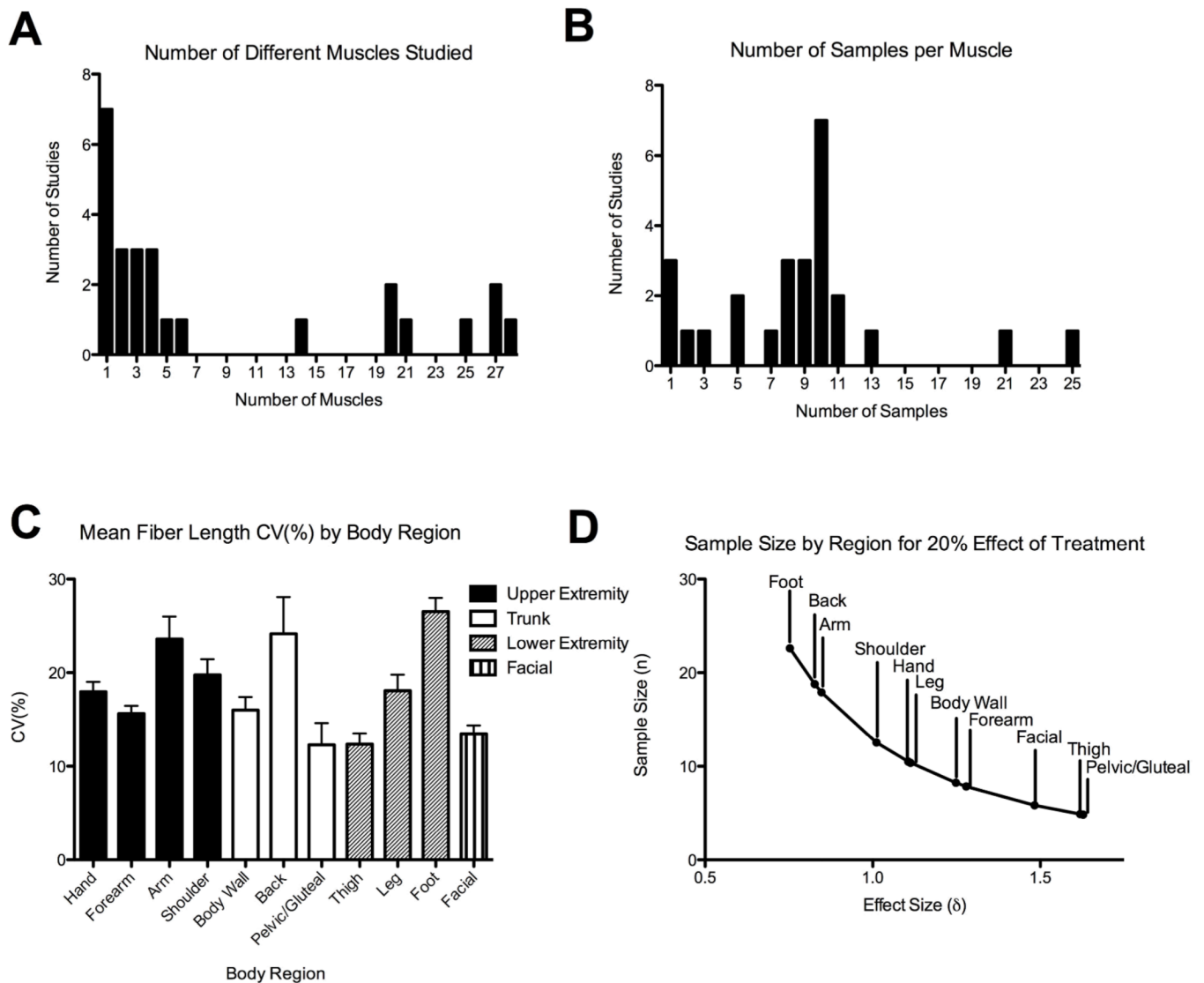
Abbreviations

CT	computed tomography
CV	coefficient of variation
MRI	magnetic resonance imaging

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**Figure 1.**

A) Histogram representation of the number of different muscles throughout the body that were measured per study B) Histogram representation of the samples studied per muscle. This typically corresponds to the number of cadaveric specimens C) Mean coefficient of variation (CV; see text for details of calculation) grouped by body region; Error bars in this graph indicate SEM. D) Plot of effect size and sample size by anatomical region estimating a 20% treatment effect.