



The Contributions of Extracellular Matrix and Sarcomere Properties to Passive Muscle Stiffness in Cerebral Palsy

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Cerebral palsy results from an upper motor neuron lesion and significantly affects skeletal muscle stiffness. The increased stiffness that occurs is partly a result of changes in the microstructural components of muscle. In particular, alterations in extracellular matrix, sarcomere length, fibre diameter, and fat content have been reported; however, experimental studies have shown wide variability in the degree of alteration. Many studies have reported changes in the extracellular matrix, while others have reported no differences. A consistent finding is increased sarcomere length in cerebral palsy affected muscle. Often many components are altered simultaneously, making it difficult to determine the individual effects on muscle stiffness. In this study, we use a three dimensional modelling approach to isolate individual effects of microstructural alterations typically occurring due to cerebral palsy on whole muscle behaviour; in particular, the effects of extracellular matrix volume fraction, stiffness, and sarcomere length. Causation between the changes to the microstructure and the overall muscle response is difficult to determine experimentally, since components of muscle cannot be manipulated individually; however, utilising a modelling approach allows greater control over each factor. We find that extracellular matrix volume fraction has the largest effect on whole muscle stiffness and mitigates effects from sarcomere length.

Keywords: skeletal muscle, extracellular matrix, finite element method, cerebral palsy, muscle stiffness, continuum model, biomechanics

1. INTRODUCTION

Cerebral palsy (CP) results from an upper motor neuron lesion and has a significant effect on the musculoskeletal system. It develops during early childhood and leads to muscle alterations including contracture, which is the chronic shortening of a muscle. Contracture results in muscle that cannot be stretched through its typical range of motion due to an increase in stiffness, and this has substantial effects on the ability of muscle to generate force and reduces daily functioning. Typically, this is observed in the upper and lower limb flexor muscles. CP will affect individuals differently, and the changes that can occur will vary depending on the location of the muscle and disease severity (de Bruin et al., 2014; Handsfield et al., 2016; Lieber and Fridén, 2019). This variability increases the difficulty in quantifying the amount and types of changes that occur as a result of CP. However, despite the variability, alterations in the microstructural properties of

skeletal muscle are commonly observed (Tisha et al., 2019), which will have a significant effect on whole muscle behaviour, including force production and movement.

There are many structural differences comparing CP muscle to typically developed (TD) muscle, including changes in fat content (Ohata et al., 2009; D'Souza et al., 2020), extracellular matrix (ECM) stiffness (Lieber et al., 2003; Smith et al., 2011), amount of ECM (Lieber et al., 2003; Smith et al., 2011), fascicle length (Mohagheghi et al., 2008), fibre diameter (Mathewson et al., 2014a), fibre geometry (Barrett and Lichtwark, 2010), and sarcomere length (Lieber and Fridén, 2002; Smith et al., 2011; Mathewson et al., 2014a). Experimental studies have investigated CP muscle stiffness *in vivo* and have found stiffer tissue compared to TD muscle using shear wave elastography (Brandenburg et al., 2016; Lee et al., 2016) and through measuring joint movement (Barber et al., 2011; van der Krogt et al., 2016). However, these methods are unable to capture the underlying causes of this increased stiffness.

The exact microstructural changes that alter whole muscle stiffness have yet to be fully understood, as the extent of measured changes varies between studies (Lieber and Fridén, 2019). For example, Smith et al. (2011) performed passive mechanical experiments on both muscle fibre bundles and single fibres extracted from CP and TD muscle. They found that CP muscle had longer *in vivo* sarcomere lengths and increased fibre bundle, but not fibre, stiffness, which suggests that the changes in muscle stiffness are due to alterations in the ECM. Another study by Mathewson et al. (2014a), which used a similar experimental protocol as Smith et al. (2011), also showed increased *in vivo* sarcomere lengths. However, the authors demonstrated a difference in the single fibre stiffness and not the fibre bundles, suggesting that there is not a significant effect from the ECM, and that any alterations to passive stiffness occur on the muscle fibre level. Smith et al. (2011) performed studies on the Gracilis and Semitendinosus muscles, whereas, Mathewson et al. (2014a) looked at the Gastrocnemii and the Soleus muscles. Mathewson et al. (2014a) mention that the difference in the results is possibly due to the locations of the muscles or different mechanical properties of ECM between TD and CP muscle. Another possible explanation for the differences between the two studies is the that the TD groups in the study by Mathewson et al. (2014a) had a much older average age (47.7 ± 15.3) compared to Smith et al. (2011) (15.8 ± 1.8). Other studies have reported that CP muscle has a greater accumulation of fibrotic tissue, and potentially even results in an ECM with a larger volume fraction but compromised stiffness (Lieber et al., 2003). Additionally, Booth et al. (2001) suggested that collagen plays a role in the increased muscle stiffness that is observed in CP. However, it has been observed that fibrosis does not always alter the stiffness of muscle (Smith and Barton, 2014), and so there may be an effect from the stiffness and structure of the ECM. In TD muscle the ECM has been shown to be a major contributor to passive whole muscle mechanics due its composition of stiff collagen fibres (Gillies and Lieber, 2011; Meyer and Lieber, 2018). In particular, work has shown that in mammalian muscle, the ECM is responsible for about half of load bearing in passive tension (Meyer and Lieber, 2018). Sarcomere length is a commonly

observed alteration in CP muscle, and has been said to have a large effect on active muscle mechanics (Lieber and Fridén, 2002; Smith et al., 2011; Mathewson et al., 2014b). While many changes have been observed in CP muscle, the changes that are most common between studies are changes in ECM volume fraction, ECM stiffness, and sarcomere length. However, the individual roles of the ECM properties and sarcomere length in passive whole muscle stiffness have yet to be fully understood.

The purpose of this study was to determine which microstructural change occurring with CP has the largest contribution to whole muscle stiffness. In particular, whether the ECM, through changes in volume fraction or stiffness, or the sarcomere, through increases in length that result in an increased passive response from the titin, will have the greatest influence on passive whole muscle behaviour. During experimental studies, it is not possible to change a component of muscle, while keeping all other components constant; therefore, causation cannot be determined. This is especially difficult in CP where many components of muscle are known to vary between individuals (Lieber and Fridén, 2019; Tisha et al., 2019). In this study, we try not to determine the cause of changes to the microstructure, but the relationship between changes to the microstructure and the overall muscle stiffness. Using a modelling approach, we investigated the influence of the microstructural components on passive muscle stiffness. We utilised a three dimensional continuum model of skeletal muscle, developed in previous studies (Rahemi et al., 2014; Ross et al., 2018b; Wakeling et al., 2020; Konno et al., 2021), which can be modified to incorporate the effects of ECM and passive fibre properties on whole muscle mechanics. Here we do not explicitly model the process of contracture, but instead the resulting changes to the microstructure. Any mechanical response we observe in this study will also be relevant to muscle without clear contracture, but with similar changes to the material properties. By modifying the material properties in the muscle, we investigated changes that occur with CP to understand how each component contributes to whole muscle stiffness.

2. METHODS

2.1. Computational Model

In this study, we utilised a continuum mechanical model of muscle as a fibre-reinforced composite biomaterial. The model uses a three field formulation in terms of the velocity, \mathbf{u} , pressure, p , and dilation, J , and the balance of strain-energy potentials based on work by Simo and Taylor (1991) and Weiss et al. (1996). In particular, we want to minimise the total strain-energy

$$E_{\text{tot}}(\mathbf{u}, p, J) = U_{\text{int}}(\mathbf{u}, p, J) - W_{\text{ext}}(\mathbf{u}), \quad (1)$$

where U_{int} is the internal strain-energy potential and W_{ext} is the total external work done on the muscle. We characterised the passive mechanical behaviour of muscle in terms of its stress-strain behaviour, which is the relationship between the stress applied to the muscle and the strain experienced. Here we use the full Cauchy stress tensors, so the shear stress response is built into this tensor implicitly and has been investigated in Konno

et al. (2021). The relationship between the stress in the material, σ , and the strain-energy potential of a material, U , is given by the constitutive law

$$\sigma = 2J^{-1}\mathbf{B}\frac{\partial U(\mathbf{B})}{\partial \mathbf{B}}, \quad (2)$$

where \mathbf{B} is a strain tensor measuring the deformation. To capture muscle, we split the model into a three dimensional isotropic base material with one dimensional fibres running along the length of the muscle, making the composite material anisotropic. The total stress response from the muscle (σ_{muscle}) is then additive contributions from the base material (σ_{base}) and fibre (σ_{fibre}) components

$$\sigma_{\text{muscle}} = \sigma_{\text{fibre}} + \sigma_{\text{base}}. \quad (3)$$

Our model is a homogenized muscle material that included contributions from the fibres and base material at every element in the mesh. A more precise formulation of the model is described in the appendix of Wakeling et al. (2020) and in Konno et al. (2021). We used a finite element method to solve the continuum model that was implemented using an open source finite element library deal.II (Arndt et al., 2017).

There are two main components of our muscle model: the fibre components and the three base material. The base material encompasses effects from extracellular matrix and cellular components, including satellite cells and capillaries, while the fibre component runs along the length of the muscle and contains the passive effects from titin and active effects from the contractile elements. To investigate the role of CP on whole muscle stiffness, considering the individual effects from the ECM and cellular components is necessary. To do this, we let α be the volume fraction of the ECM, which includes effects from the collagen fibre matrix (Figure 1). Meanwhile, $1 - \alpha$ is the volume fraction of the cellular component, which includes effects from any other material in the muscle aside from the contractile units. In particular the cellular material includes the myofibres, satellite cells, and other cellular materials. We also introduced a parameter s_{ECM} (Figure 1), which is a stiffness factor multiplying the stiffness of ECM. A larger s_{ECM} corresponds to a stiffer ECM, which can occur as a result of structural changes while the ECM volume fraction remains the same (Gillies et al., 2016). Meanwhile, a smaller value of s_{ECM} results in an ECM with decreased stiffness, which can occur as a result of an ECM with compromised structure (Lieber et al., 2003). The total stress response from the base material is a homogenization of the ECM and cellular components given by

$$\sigma_{\text{base}}(\mathbf{B}) = \alpha s_{\text{ECM}}\sigma_{\text{ECM}}(\mathbf{B}) + (1 - \alpha)\sigma_{\text{CELL}}(\mathbf{B}). \quad (4)$$

$\sigma_{\text{ECM}}(\mathbf{B})$ corresponds to the stress response from the ECM, while $\sigma_{\text{CELL}}(\mathbf{B})$ is the stress response from the cellular component. To ensure the volume of the muscle remains nearly constant, the bulk modulus for the cellular component is chosen to be 1×10^7 Pa, while the bulk modulus of the ECM component was set to 1×10^6 Pa (Konno et al., 2021). While the cellular component of the muscle contributes less to the overall stress response, it consists largely of water, so its bulk modulus is set to be larger than the

ECM component. The exact form of the stress-strain response for the microstructural components are given in Konno et al. (2021) along with more detail on the homogenized base material.

The other alteration typically observed in CP is an increase in *in vivo* sarcomere length (Smith et al., 2011; Mathewson et al., 2014b), which alters the passive muscle stiffness by stretching the titin protein. Experimentally, the sarcomere lengths for muscles in the lower extremities were measured with 90 degrees hip and knee flexion (Smith et al., 2011; Mathewson et al., 2014a), and with the ankle in full dorsiflexion for the lower leg muscles (Mathewson et al., 2014a). Our model does not have a joint angle, and so we define the *in vivo* length of the sarcomeres to be the length of the sarcomeres when the whole muscle is at its resting length. This change in length also decreases the contractile force produced when the muscle is active by reducing the number of attached actin-myosin crossbridges (Figure 1). We modelled this using a dimensionless parameter, c_{sarco} , which corresponds to a shift in the passive force-length curve of the sarcomeres

$$\sigma_{\text{fibre}} = \sigma_{\text{fibre}}(\bar{\lambda}_{\text{tot}} + c_{\text{sarco}}), \quad (5)$$

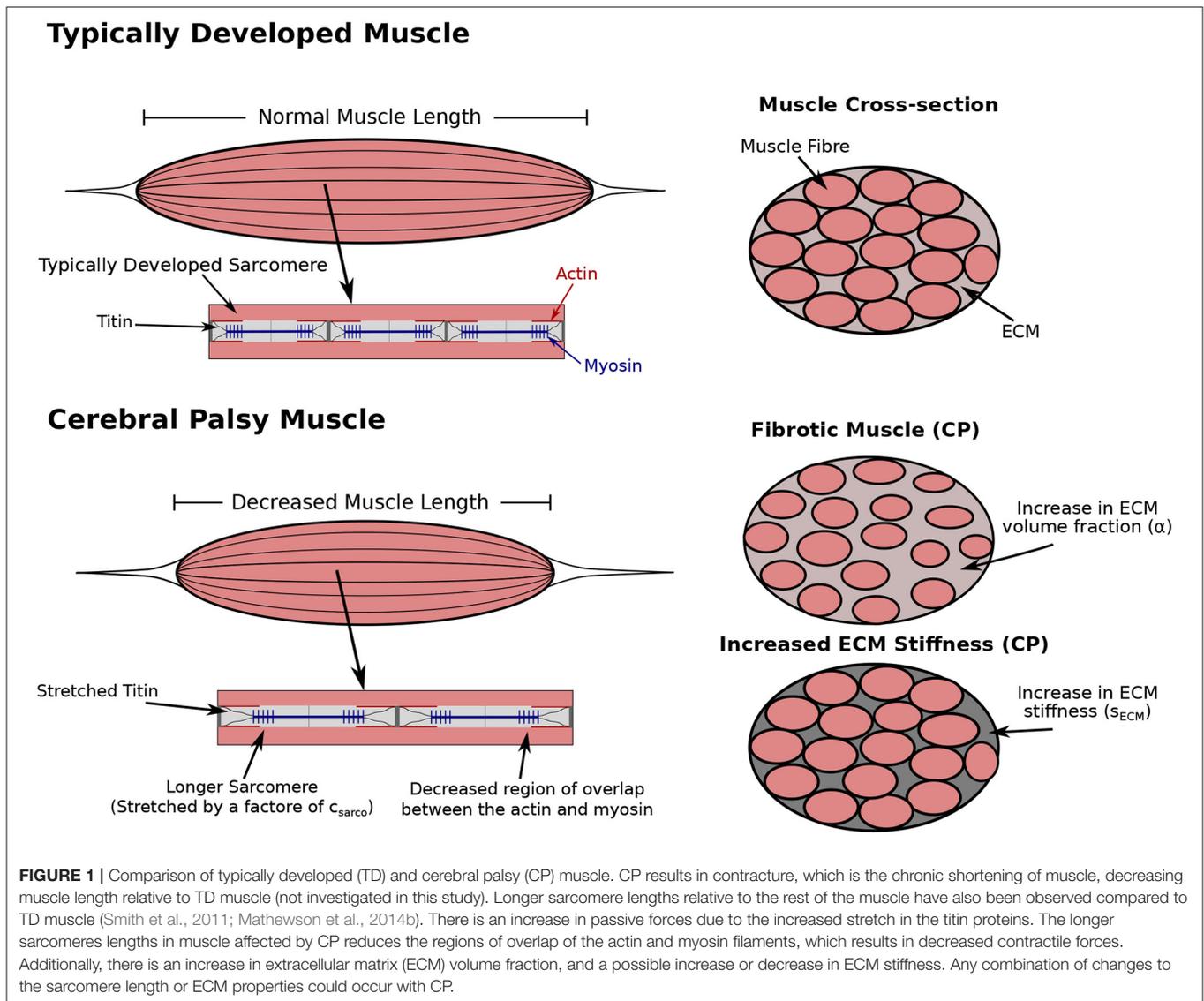
where $\bar{\lambda}_{\text{tot}}$ is the total average stretch of the fibres over the muscle volume. This parameter acts as an additive contribution to the intrinsic stress-stretch relationship, resulting in larger stresses in the fibres at a given stretch (Figure 2). It is important to note that, while the fibre component of the muscle depends on c_{sarco} , the intrinsic stress response from the base material, $\sigma_{\text{base}}(\mathbf{B})$, only depends on the deformation and stretch of the muscle, and not c_{sarco} . At a value of $c_{\text{sarco}} = 0.0$, the behaviour of the sarcomere is the same as that of TD muscle. Increasing values of c_{sarco} results in longer lengths of the sarcomeres given by

$$l_{\text{sarco}} = l_0(\bar{\lambda}_{\text{tot}} + c_{\text{sarco}}), \quad (6)$$

where l_{sarco} is the new length of the sarcomere and we assume $l_0 = 2.2\mu\text{m}$ is the optimal length of a sarcomere (Burkholder and Lieber, 2001). This will vary depending on the value of c_{sarco} . The fibres in the model are based off the one dimensional Hill type model (Hill, 1938; Zajac, 1989) and are described in Wakeling et al. (2020). It is possible the behaviour of the fibre will vary in more than a shift of its force-length curve, such as a different force-length relationship, which may be due to factors in the myofibres other than the sarcomeres. However, as this is not observed frequently and data are limited, we focus on the effects from the sarcomere length.

2.2. Whole Muscle Experiments

To investigate the passive effects of α , s_{ECM} , and c_{sarco} on skeletal muscle, we constructed a rectangular block of muscle with dimensions 3 cm \times 1 cm \times 1 cm. These dimensions, while not the same as muscle affected by CP, sufficiently capture the behaviour of muscle on a macroscopic scale. Using a block geometry reduces the need to consider the additional effects from architecture, aponeurosis, and pennation angle, which affects muscle behaviour (Wakeling et al., 2020), and instead allowed us to isolate the effects due to CP independent of a specific architecture. Additionally, this geometry has been previously



validated to capture the general qualitative behaviour of muscle when compared to the mechanics within a MRI derived whole muscle geometry (Wakeling et al., 2020) and has the benefit of computational simplicity. To compare the passive behaviour of the model to experimental data, we performed stress-strain tests. This involved constraining one end face of the model from movement in all directions, while a normal stress was applied to the opposite face stretching the muscle. In addition to the stress-strain tests, we investigated the stiffness of the muscle to compare with experimental studies (e.g., Smith et al. (2011); Mathewson et al. (2014b)). α , s_{ECM} , and c_{sarco} each have an individual contribution to the overall stiffness of muscle. To investigate the stiffness in the model, the modulus (in Pa) of the muscle material was calculated during the stress-strain experiments using the slope of the tangent line to the overall stress-stretch relationship. This was done by performing a nonlinear least-squares fit of a cubic polynomial to the overall stress-strain data in the

longitudinal direction. This method for calculating the modulus is only representative of the stiffness at the given stretch value, since the stress-stretch curves are nonlinear; however, we do this to compare with experimental studies.

TD muscle has been observed to have a value for α between 0.02 and 0.10 (Binder-Markey et al., 2020), while larger volume fractions ($\alpha \approx 0.6$) have been observed for fibrotic tissue (Lieber et al., 2003; Smith and Barton, 2014). Experimental studies have only found an increase in *in vivo* sarcomere lengths (Lieber and Fridén, 2002; Smith et al., 2011; Mathewson et al., 2014a), so we varied c_{sarco} from 0 to 0.75. This corresponded to a 0–75 % increase in the sarcomeres relative to the sarcomeres in typically developed muscle, which has been observed in the literature (Lieber and Fridén, 2002; Smith et al., 2011; Mathewson et al., 2014b). Experiments are often performed on severe cases of CP, so larger sarcomere lengths have been reported ($c_{sarco} > 0.75$); however, in this study, we considered less severely stretched

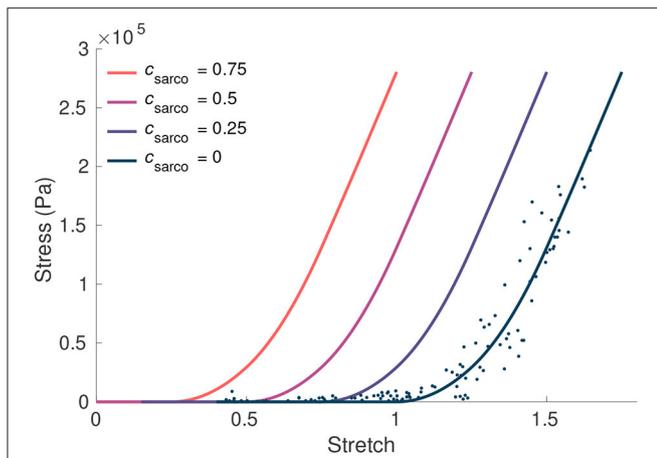


FIGURE 2 | The influence of c_{sarco} on the intrinsic fibre stress-stretch relationship. Here we plot the stress from the fibre component of our model against the stretch in the fibres. The curve corresponding to typically developed muscle ($c_{\text{sarco}} = 0$) was obtained based on curves and data in Ross et al. (2018a). This was done through trigonometric polynomial and second-order piecewise polynomial fits to experimental data from Winters et al. (2011) (denoted by the dots).

sarcomeres to represent less severe cases of CP. It is possible that muscle altered by CP does not always have such a substantial increase in sarcomere length, since measurements are typically taken from children with severe CP undergoing surgery (Lieber and Fridén, 2019). The final parameter that was manipulated in the model is s_{ECM} . While the stiffness of muscle can vary depending on the type of muscle, the properties of the base material, including the effects from the ECM, represent and have been validated for TD muscle (Konno et al., 2021), so we set $s_{\text{ECM}} = 1$ for TD muscle (this corresponds to a value of 150 in Konno et al., 2021). During our stress-strain tests, we then considered the possibility of the ECM component of muscle being stiffer ($s_{\text{ECM}} = 1.33$) and less stiff ($s_{\text{ECM}} = 0.66$). Note that a s_{ECM} value of 1.33 corresponds to a stiffness of 133 % compared to TD muscle, while a value 0.66 corresponds to a stiffness of 66 % relative to TD muscle. Data for the changes in stiffness of the ECM are not available experimentally, so these values of s_{ECM} were chosen to investigate the effects of altering this component. In summary, to investigate the effects of CP, our parameter ranges were $\alpha = 0.02$ to 0.6, $c_{\text{sarco}} = 0.0$ to 0.75, and $s_{\text{ECM}} = 0.66$ to 1.33, while TD muscle had parameters $\alpha = 0.05$, $c_{\text{sarco}} = 0.0$, and $s_{\text{ECM}} = 1.0$.

3. RESULTS

3.1. Effects of c_{sarco}

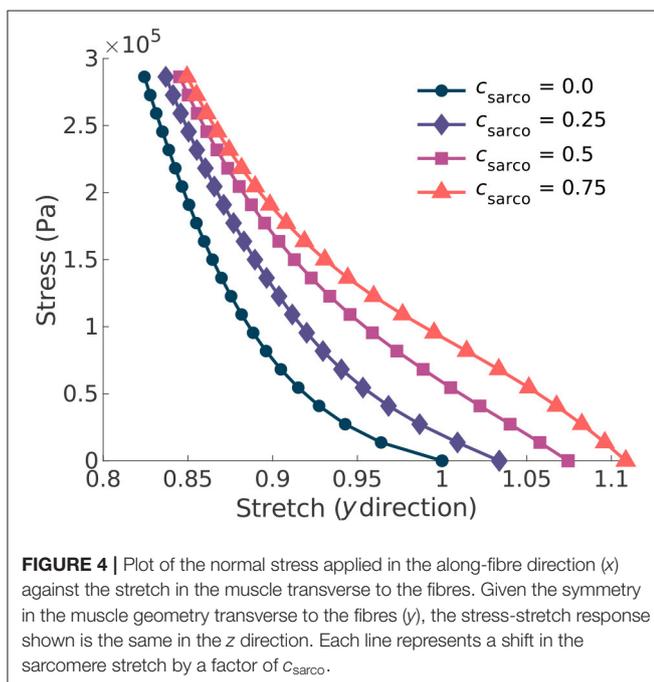
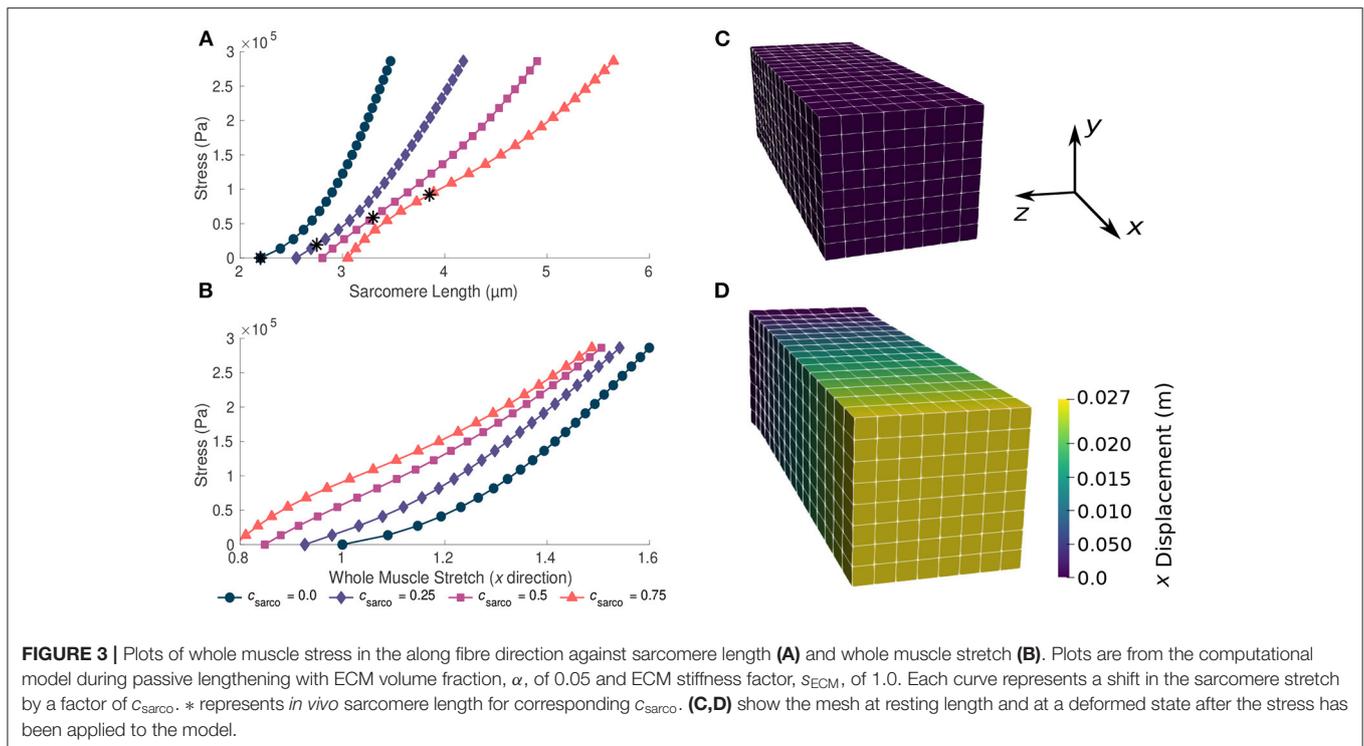
For TD muscle ($c_{\text{sarco}} = 0.0$, $s_{\text{ECM}} = 1.0$, and $\alpha = 0.05$), we observed typical overall stress-stretch behaviour for passive skeletal muscle: as the stress increased, the muscle stretch and sarcomere lengths also increased (Figure 3). The muscle block in its resting and stretched states is shown in Figures 3C,D,

respectively. The shift in the intrinsic passive sarcomere stress-stretch relationship, c_{sarco} , affected the muscle behaviour in both the stress-length for the sarcomeres and overall stress-stretch for the whole muscle relationships of the model (Figure 3). At *in vivo* lengths, we found that there is no longer zero stress for $c_{\text{sarco}} > 0.0$ (Figure 3A). This indicates that larger forces are required to stretch muscles with increased sarcomere lengths, as well as to hold it at the resting length of the muscle. We also see that there is a nonlinear relationship between the effect of c_{sarco} on the fibre component (Figure 2) and the overall muscle response which is influenced by the base material (Figure 3). Additionally, optimal length of the sarcomeres no longer occurred at the same resting length of the whole muscle. For the same range of stress values ($0-3 \times 10^5$ Pa), we saw a larger range of whole muscle stretches with larger c_{sarco} (Figure 3). This is likely due to effects from the base material and, therefore, the ECM, which acts to deform the muscle back to optimal length. At stretch values less than 1.0, the base material works to extend the muscle to optimal length, while the sarcomeres are still working to shorten the muscle for $c_{\text{sarco}} > 0.0$.

In addition to the behaviour in the along-fibre direction of the muscle, c_{sarco} also affects the behaviour transverse to the muscle fibres (Figure 4). We observed a similar change in concavity of the stress-stretch curves in both the stress-stretch relationships in the longitudinal (Figure 3B) and transverse (Figure 4) directions. This demonstrates similar effects from the muscle ECM component in both directions. For stretch values in the y direction less than 0.85, the influence of the sarcomeres on the stress-stretch relationship decreased, and there was larger influence from the ECM. For smaller normal stresses in the longitudinal directions, there were larger effects from sarcomere length relative to larger stresses (Figure 4). The sarcomeres, acting only in the along-fibre direction, altered three dimensional deformation, which could affect muscle force production (Wakeling et al., 2020).

3.2. Effects of α and s_{ECM}

The ECM properties also had a substantial effect on the overall stress-stretch relationships (Figure 5). Given the range of possible values for the ECM volume fraction, α , (0.02–0.6), it had a larger effect on the muscle stiffness compared to the ECM stiffness parameter, s_{ECM} . As s_{ECM} was increased, with fixed $\alpha = 0.05$ or 0.6, the overall stress-stretch relationship became more linear and covered a smaller range of stretch values (Figure 5). Increases in both α and s_{ECM} reduced the effect from the sarcomere length on the stress-stretch relationship (Figure 5). Due to the lack of available data for the stiffness of the ECM, the s_{ECM} was only varied between 0.66 and 1.33, which is a relatively small range compared to the volume fraction of the ECM. Similar effects were observed from changing s_{ECM} and α , but α had more effect on the overall stress-stretch behaviour. This is expected given the change in composition of the base material; however, due to the limited data for s_{ECM} , it is possible that real muscle has a larger range of values than investigated in this study, but to ensure that we remain within realistic physiological ranges we chose this limited range. Our results showed that s_{ECM} had very little effect on the overall stress-stress

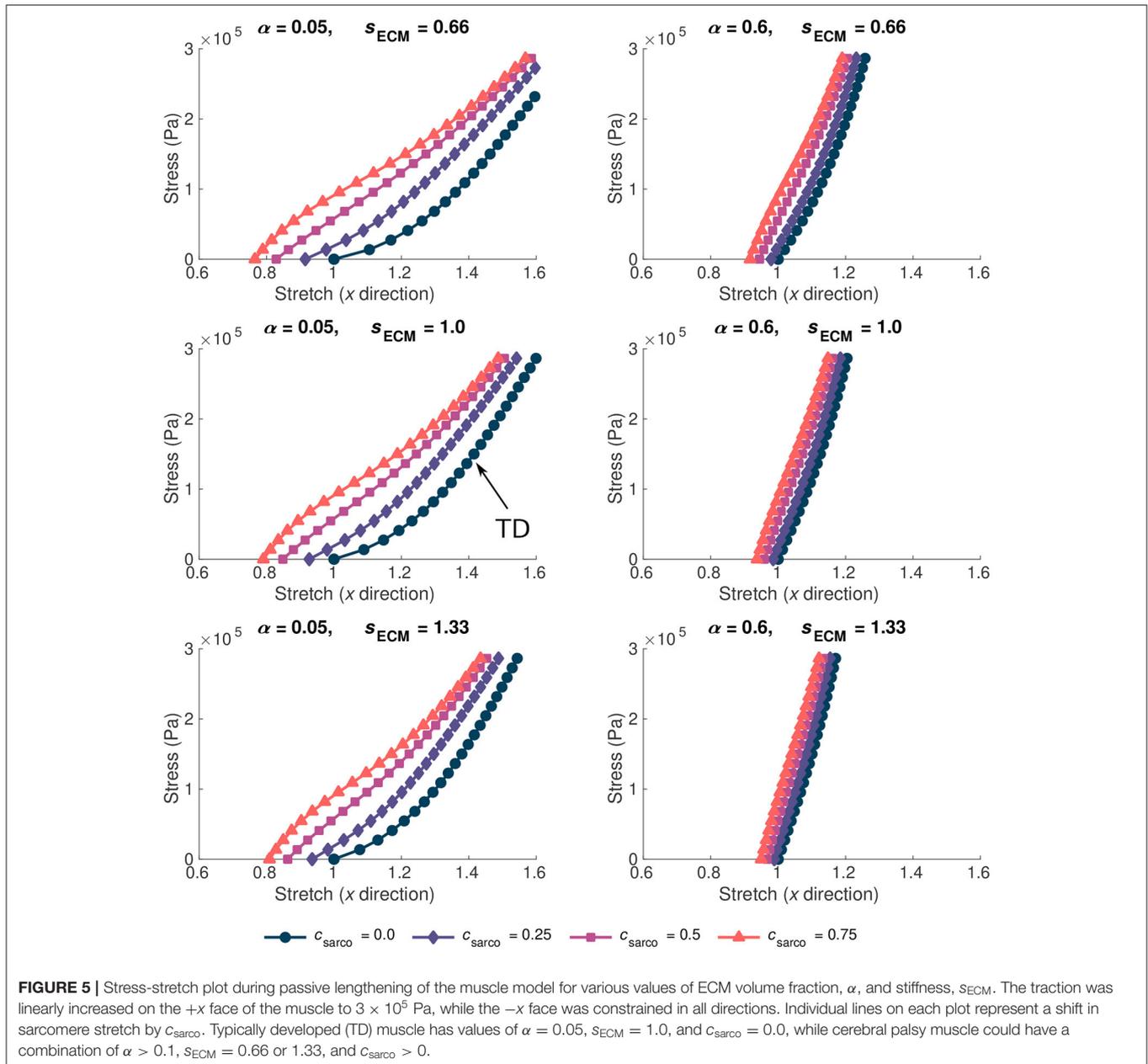


relationship for smaller values of α and it has the most effect when α is large. The main effects from the s_{ECM} are in altering the influence of c_{sarco} on the overall muscle stress-stretch relationship (Figure 5). Varying s_{ECM} over a larger range of values would likely only influence the effect of the sarcomere length on the overall stress-stretch response.

3.3. Muscle Stiffness

As c_{sarco} was increased up to a value of 0.5, the modulus of the muscle in the x direction increased at optimal length of the muscle (Figures 6A,B). However, after a value of 0.5, the muscle modulus decreased, and this was observed when looking at the variations in c_{sarco} with constant α and s_{ECM} (Figures 6A,B). The modulus at optimal length was dominated by α . For changes in c_{sarco} and s_{ECM} , the change in modulus (at most 4×10^5 Pa) was less than the possible variation in modulus with changes in α (up to 15×10^5 Pa for highly fibrotic tissue). While holding c_{sarco} constant, we found a linear increase in the modulus when increasing α and s_{ECM} (Figures 6C,D).

While holding c_{sarco} constant, there was a larger effect from volume fraction of the ECM, α , than the stiffness of the ECM, s_{ECM} , on the overall muscle stiffness (Figures 7A,B). However, as α was increased, there was a greater effect of s_{ECM} . The nonlinear behaviour, which showed increasing muscle modulus with increasing α and s_{ECM} was more pronounced at larger stretches (Figures 7B,D). At a stretch of 1.20 in the x direction, the stiffness appeared to be more nonlinear when moving along the lines of constant s_{ECM} and when moving along the lines of constant α for $\alpha > 0.2$ (Figures 7C,D). When holding s_{ECM} constant, there was a larger effect α on the modulus of the muscle compared to c_{sarco} , particularly at larger stretch values (Figures 7C,D). As the stretch increased there was an increase in modulus from the ECM parameters; however, there was a decrease in the effects of c_{sarco} (Figures 7E,F). The reduced influence of c_{sarco} was due to more pronounced behaviour from the base material at larger stretches (Figures 5, 7).



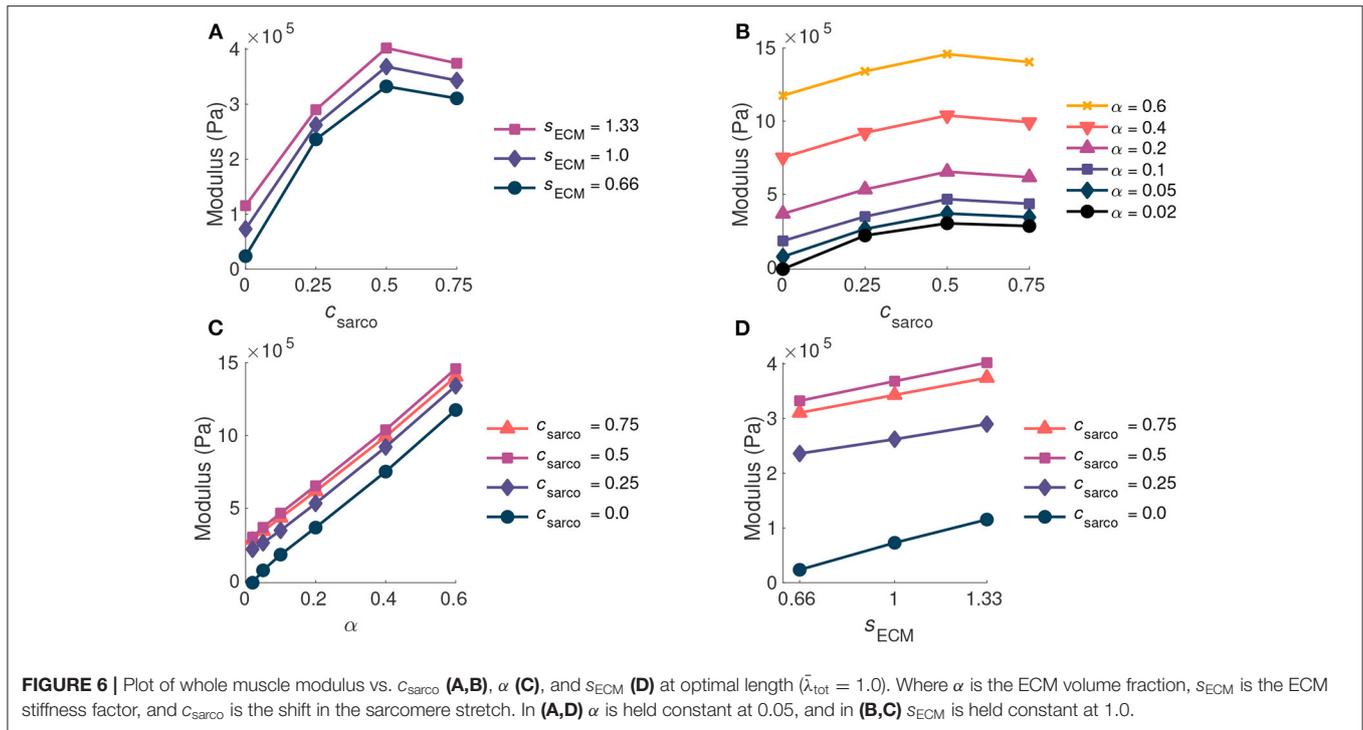
4. DISCUSSION

In CP, alterations occur on the microstructural level that can influence whole muscle stiffness and reduce function. In particular, alterations to ECM properties and sarcomere length can occur; however, their relative contributions to muscle stiffness in CP is unknown. Isolating individual effects on passive muscle stiffness is difficult to do in experimental studies as there is large variability between subjects and individual muscles (Calvo et al., 2010; Takaza et al., 2013; Mohammadkhan et al., 2016). Therefore, to determine whether the ECM properties or sarcomere lengths have more effect on the passive muscle behaviour, we used a three dimensional continuum model

(Rahemi et al., 2014; Ross et al., 2018b; Wakeling et al., 2020; Konno et al., 2021). This model does not actually develop joint contractures; however, it allows us to isolate the effects from individual microscopic components, and investigate the relative contributions to whole muscle function.

4.1. Physiological Changes to Muscle During Cerebral Palsy

The ECM is composed of a highly structured arrangement of collagen fibres and plays a substantial role in skeletal muscle mechanics (Gillies and Lieber, 2011). In this study, we investigated the effects of changes to ECM volume fraction and



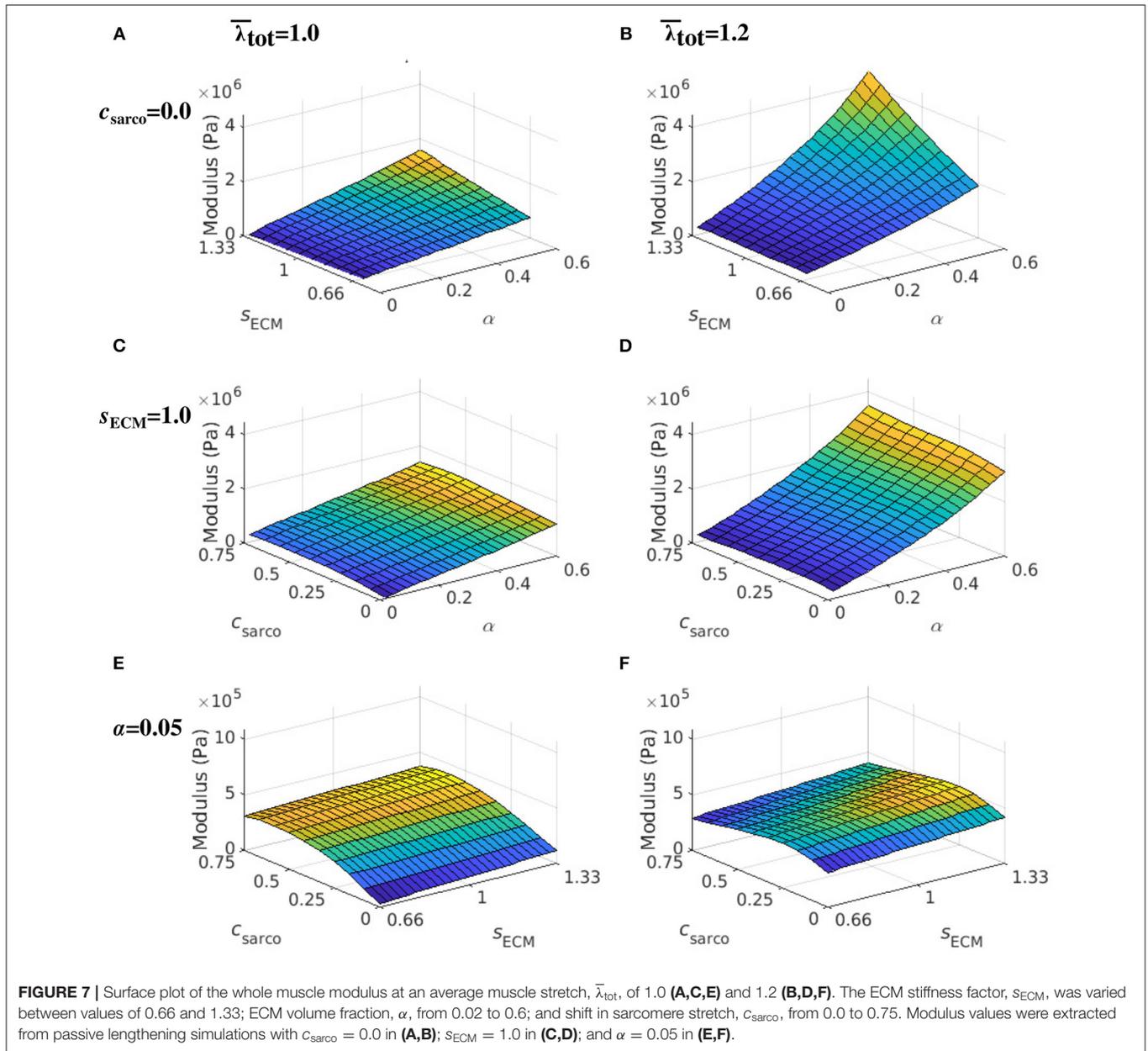
stiffness on the whole muscle stiffness. Changes in ECM volume fraction have been observed in previous studies, particularly as a result of fibrosis (eg. Smith et al. (2011)). This corresponds to an increase in ECM material, while the contributions from the cellular components in muscle, such as the contractile fibres and other cells, decreases. In addition, fibrosis creates a physical barrier that can impact muscle regeneration (Chen and Li, 2009), which will reduce the ability for muscle to grow and add sarcomeres in the muscle fibres, further decreasing the compliance of the muscle. Additionally, it is possible that while the volume fraction stays constant, changes in the structure or composition of collagen types varies. However, studies have found that the ratio of collagen isoforms is the same in both TD and CP muscle (Smith et al., 2019), and so it is unlikely that a difference in collagen isoforms in muscle accounts for the increase in whole muscle stiffness with CP. It is possible that there are alterations in ECM structure, such as the organisation of collagen fibres, that occur with CP (Lieber et al., 2003), and this could increase or decrease ECM stiffness depending on the change. Therefore, both effects were considered in the model to investigate the relative contributions to stiffness on a whole muscle level.

It has also been well documented that increases in the sarcomere length occur with CP (Lieber and Fridén, 2019; Tisha et al., 2019). Stiffness changes have been reported on the fibre level by looking at the stress-strain relationship for TD and CP muscle fibres (Mathewson et al., 2014a). It is possible that these effects are not only a result of increased sarcomere lengths, but due to different titin isoforms, as they could result in a different stress-strain relationship for the individual sarcomeres (Prado et al., 2005). However, Smith et al. (2011) found that

there is no change in the composition of titin isoforms between TD and CP muscle. Therefore, changes in stiffness due to the sarcomeres are not likely due to changes in titin isoforms. While it is possible that an increased stretch of the titin is responsible for the increased passive stiffness of the fibres, this could also be a result of changes to other mechanical properties in the myofibres. More investigation is required to confirm the main cause of the increased stiffness at a fibre level.

4.2. Microstructural Contributions to Whole Muscle Behaviour

It has been demonstrated experimentally that the ECM has a significant contribution to muscle passive stiffness (Gillies and Lieber, 2011), and that fibrosis has been observed in CP (Lieber et al., 2003). We found that the volume fraction of the ECM had a larger influence on whole muscle stiffness compared to ECM stiffness and sarcomere length. The contribution from the ECM increased as stretch increased (Figure 7), demonstrating a nonlinear relationship between the ECM volume fraction and muscle stretch. At larger stretch values, the ECM contributes more to the whole muscle stiffness; these nonlinear effects imply that fibrosis will substantially reduce the ability of a muscle to deform at larger stretch values. The ECM is composed of crimped collagen fibres, which likely do not contribute as much to the stress initially (Gillies and Lieber, 2011), and this is reflected in a smaller effect from the ECM volume fraction at optimal length. Currently, experimental data for the variation in stiffness of the ECM due to structural changes are not available; however, in the ranges tested in this study, the stiffness of the ECM did not alter whole muscle stiffness as much as the volume fraction. At larger volume fractions of the ECM there was a more substantial



contribution from the ECM stiffness (**Figure 7B**), and since larger volume fractions are typically seen in CP, this could play a larger role.

The contribution of the sarcomere length to whole muscle stiffness varied depending on the ECM properties. There was minimal effect of the sarcomeres on the passive stiffness in the fibrotic tissue (**Figure 6**), which corresponds to volume fractions of ECM greater than 10%, and this larger effect from the ECM has been observed during experiments (Smith et al., 2011). Furthermore, the sarcomere effects are mitigated at larger stretches as the ECM begins to dominate the muscle stiffness. At whole muscle stretches near 1.0, we found a larger effect of sarcomere length (**Figure 6**), which agrees with the results

from Mathewson et al. (2014a) for fibre bundles. The work by Mathewson et al. (2014a) indicates that there was no increase in the stiffness of the ECM during CP in the muscles investigated. They found that there was no difference in the stiffness of fibre bundles at larger stretches, which could be explained by the dominating behaviour of the ECM. This agrees with our findings that demonstrate that if a muscle is operating near optimal length, then there might be a noticeable effect of sarcomere length. However, if the muscle has a larger range of motion, then the ECM would likely have a larger contribution to muscle passive stiffness. It is likely that the lengthening of the sarcomeres during CP has a larger effect on the active properties of the muscle (which we have not evaluated in this study)

compared to the passive properties as noted by Lieber and Fridén (2019).

Using this model we can obtain a deeper understanding of the three-dimensional effects that occur in muscle altered by CP. As shown in previous modelling (Ryan et al., 2020; Wakeling et al., 2020) and experimental studies (Randhawa and Wakeling, 2018), the ability of a muscle to deform both in the along and transverse fibre directions can alter muscle function. Additionally, our results agree with experimental evidence that the whole muscle response is not the same as the response from individual fibres (Ward et al., 2020). In our model, the three-dimensional behaviour is captured in part by the base material, which works to return the muscle to its original state. At longer muscle lengths, the base material will work in the same direction as the sarcomeres, which are trying to shorten the muscle. Meanwhile, when the whole muscle stretch is less than one, the ECM will be working to return the muscle back to the original muscle length. We have observed in the model that the stiffness of the muscle decreases after sarcomere lengths greater than $3.3 \mu\text{m}$ (Figure 6), and this is due to the three dimensional behaviour of the model we are using. In a one dimensional model, there are no effects from the volume conserving nature of the base material or other effects transverse to the fibres. This is a nearly incompressible and nonlinear model, and so the effects from the volumetric component of the model contribute more with larger shifts in the sarcomere force-length curve. While these effects have been observed based on our assumptions for the model (see Wakeling et al., 2020; Konno et al., 2021), which are typical of many finite element models (Blemker and Delp, 2005; Sharafi and Blemker, 2011; Spyrou et al., 2017), these effects have not been reported experimentally. Experimentally, the decrease in muscle stiffness may not be as substantial as the changes observed in this study; however it is likely a similar trend would appear. Another important consequence of the three-dimensional behaviour is that changes occurring strictly in the along-fibre direction, such as changes in the sarcomere length, affect the stretch transverse to the fibres (Figure 4). In particular, the bulging and stretching in the transverse direction is decreased by increased *in vivo* sarcomere lengths, which increases the passive stiffness of the muscle fibres. Given this reduced movement in the transverse direction, it is likely that there would be a decreased contractile force produced given the significant effect of three dimensional deformation (Ryan et al., 2020). This demonstrates that to accurately capture all of the effects from CP, investigating three dimensional behaviour is required to completely understand the mechanical behaviour of the muscle.

4.3. Model Parameters

Experimental studies are key to understanding the mechanical changes that occur with CP; however, many of the procedures are invasive and unable to determine the exact role each change due to CP plays in altering muscle stiffness (Smith et al., 2011; Lieber and Fridén, 2019; Tisha et al., 2019). Additionally, there is contradicting data as to whether fibres, ECM, or both have a substantial contribution to passive stiffness (Smith et al., 2011; Mathewson et al., 2014a), which likely depends on the severity of the disease (Tisha et al., 2019). There are less invasive procedures

that have been developed to investigate the relationship between muscle stiffness and CP (Lee et al., 2016); however, they are still unable to isolate the role of individual factors. For example, experimental studies have found that stiffness of CP muscle is twice as high as TD muscle (von Walden et al., 2017); however, they were not able to determine which microstructural changes led to this increase in stiffness. While this model cannot directly determine which microstructural changes will cause this experimental increase in stiffness, it can provide insight into how various changes on the microscopic level could lead to these effects on muscle stiffness. We have observed that there is approximately double the increase in stiffness when the volume fraction of ECM in our model increases from 5 to 20%. Another possible way to achieve this increase in muscle stiffness is through increasing the stiffness of the ECM, or some combination of the two. The possible changes that cause increased stiffness can be investigated through our modelling approach and can indicate which factors may have the most impact on muscle behaviour. It is difficult to perform experimental tests on whole muscles affected by CP, although tests have been done on mice with spasticity or fibrosis (Ziv et al., 1984; Smith and Barton, 2014), as muscle can only be dissected during surgery making it difficult to obtain data for an accurate comparison to similar TD muscle tissue. Muscle is a three-dimensional material, so applying a continuum model to CP muscle allows us to understand the underlying muscle mechanics. In particular, developing an understanding of the complete behaviour of muscle will give insight into the role each microstructural alteration that occurs in CP will play in whole muscle mechanical behaviour.

While the model has the ability to investigate behaviour of muscle that is difficult to examine experimentally, it relies on accurate experimental data for its intrinsic properties. Unfortunately, mechanical data for the effects of stiffness of the ECM are not available, so the value for the ECM stiffness parameter was chosen to vary by 33% from healthy muscle. It is possible that changes in the structure of the ECM would change by more than this value; however, these values were chosen to probe the behaviour of the ECM stiffness. Given the derivation of the whole muscle stress in the model (Equation 4) it is likely that the volume fraction of the ECM would still have the largest contribution to whole muscle stiffness. Both the ECM volume fraction and stiffness multiply the ECM stress response, so they have similar contributions to whole muscle behaviour for small variations in their values. However, only the ECM volume fraction decreases the contributions from the cellular components. This aspect of the model is realistic, since it is not likely changes in the structure of the ECM will decrease the contribution of the fibres to whole muscle stiffness.

4.4. Limitations and Future Directions

A limitation of this model is the lack of current experimental certainty on changes that occur with CP. Many changes to individual components have been observed in CP affected muscle; however, the extent to which microstructural changes occur are varied (Tisha et al., 2019). Therefore, the effectiveness of the model in providing a comparison to CP muscle will depend on the specific muscle. Additionally, there are very little

data available for the changes in stiffness of the ECM, and so it is possible that this could vary more than investigated in this study. This would result in a large influence of the stiffness of the ECM component. Work by Brashear et al. (2021) found that stiffness and orientation of the ECM component may have more effect than the amount of the ECM, so this lack of experimental data for ECM stiffness is a limitation of our model. Additionally, it is possible that a focalized accumulation of collagen de Bruin et al. (2014); Von Walden et al. (2018) could occur in contrast to the even distribution investigated in this study. This would likely influence the mechanical response of muscle; however, this was not investigated in this study. The response of the base material likely changes in response to compression as opposed to tension. Currently, the available data for the ECM is limited to tension, and so a different response for compression was not implemented in the model. However, we expect the effect on the output from our model would be minimal.

In the model, we have assumed for simplicity that with changes in the volume fraction of the ECM, there is no effect on the amount of force produced by the fibres. Any reduction in contribution from the muscle fibres is assumed to be included in the decrease in cellular component contribution to the base material response. In addition to changes in the microstructure, it is possible that changes occur to the geometry of the muscle in CP. The results of this study only demonstrate the effects of the changes to the material properties, and the effect of changes to the geometry could be investigated in future work. We have not investigated the active behaviour of muscle in this study, although it is fundamental in muscle function. In CP, the contractile force produced has been seen to decrease (Stackhouse et al., 2005), so using this model to investigate the influence of ECM and sarcomere properties on active force would be valuable and would give additional insight into how the structural alterations that occur with CP individually impact muscle contraction. In this model, the properties of our TD muscle may not be representative of all muscles as the material properties vary both across and within studies (Calvo et al., 2010; Takaza et al., 2013; Mohammadkhah et al., 2016). So, while the qualitative passive behaviour is captured in this model, the exact values could vary between muscles. However, we would expect the general trends observed during this study to hold.

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5. CONCLUSION

The purpose of this study was to determine the effects of the microstructural changes that are normally observed during experimental studies of CP muscle, including variation in ECM volume fraction, stiffness, and sarcomere length, on whole muscle stiffness. To do this, a three dimensional computational model of skeletal muscle was used, and overall stress-stretch relationships and muscle stiffness were calculated by measuring the passive stress of the whole muscle. We found that the volume fraction of the ECM had a larger effect on overall muscle stiffness compared to the ECM stiffness and sarcomere length, and that the effects of the sarcomere length were mitigated at larger ECM volume fractions. Investigating these effects provides a causal relationship between changes to microstructural properties and the overall response of the muscle. Experimental research is currently unable to vary independent components of muscle, and so this work can be used to help direct future experimental research. In this study, we were able to determine the crucial role that the microstructural alterations observed in CP have on whole skeletal muscle behaviour.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RK carried out experimental design, performed simulations, analyses of results, and drafted and edited the manuscript. NN, JW, and SR carried out experimental design, analyses of results, and editing of the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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