Published in final edited form as:

Physiol Meas. 2013 May ; 34(5): 487–502. doi:10.1088/0967-3334/34/5/487.

Circuit Modeling of the Electrical Impedance Part III : Disuse Following Bone Fracture

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Abstract

Multifrequency measurements of the electrical impedance of muscle have been extended to the study of disuse following bone fracture, and analyzed using the 5-element circuit model used earlier in the study of the effects of disease. Eighteen subjects recovering from simple fractures on upper or lower limbs were examined (10 males, 8 females, aged 18-66). Muscles on un-injured contralateral limbs were used as comparison standards, and results are presented in terms of the ratios $p(\text{injured})/p(\text{un-injured})$, where p stands for the circuit parameter r_1 , r_2 , r_3 , $1/c_1$, or $1/c_2$. These are strikingly similar to the diseased-to-healthy ratios for patients with neuromuscular disease, reported in part I of this series. In particular r_1 is virtually unaffected and the ratios for r_2 , r_3 , $1/c_1$, and $1/c_2$ can be as large as in serious disease. Furthermore the same pattern of relationships between the parameters is found, suggesting that there is a common underlying mechanism for the impedance changes. Atrophy and fibrosis are examined as candidates for that mechanism, but it is argued that their effects are too small to explain the observed changes. Fundamental considerations aside, the sensitivity, reproducibility and technical simplicity of the technique recommends its use for in-flight assessments of muscles during orbital or interplanetary missions.

Keywords

muscle; electrical impedance; disuse; atrophy; circuit modeling

Introduction

Muscle atrophy is well known as a major consequence of normal aging, neuromuscular disease, cast immobilization, and more recently of space flight. Its physiological causes have been studied extensively using a variety of techniques, ranging from simple anthropometry to biopsy/histology to imaging. (See, e.g. the reviews by Sandri, 2008, Qin , 2010, and Gillies and Lieber, 2011.) But gauging its effects in a clinical setting often involves strength or other performance tests which risk introducing subjective factors (“central activity”), and Tarulli et al. (2009) have shown that the phase of the 50 kHz electrical impedance offers an alternative which is both objective and well suited to clinical use. The technique, electrical impedance myography (EIM), is non-invasive and requires no active patient participation, it employs available and quite simple instrumentation, and it is suitable for use at the bedside.

As its name implies, multi-frequency EIM (MFEIM) is the extension of the original single frequency technique to include a wide range of measuring frequencies. Its focus is on the interpretation of the frequency dependences of the resistance and reactance components of the impedance, $R(f)$ and $X(f)$, and two earlier papers have shown that equivalent-circuit parameterization of the frequency dependences offers reliable and sensitive indices for assessing muscle in much greater detail than can be gained from the phase alone (Shiffman and Rutkove, 2013a,b -- “Part I” and “Part II” in what follows). More importantly, they

serve as steps in testing the hypothesis that the parameters can be correlated with specific features of the underlying anatomy or physiology of the subject.

Those papers applied the method to healthy subjects and to patients with neuromuscular disease, and the present investigation asks whether MFEIM can also be profitably used to study the effects of disuse, in particular following bone fracture. As in the earlier work, the basic approach is the analysis of the impedance according to a particular 5-element circuit model, now in an abnormal but disease-free context. Beyond that, it is reasonable to expect that the study of cases where there were no deeper-lying, physiological involvements would assist in the interpretation of changes accompanying disease. To emphasize this we note that MFEIM is a sensitive and quantitative measure of the severity of *neurogenic* diseases, and it seems far more likely that the associated impedance changes reflect secondary effects rather than underlying neurological conditions. (The volume of nerve tissue is far too small to affect the measured impedance directly.) One might argue further that what is found for all neuromuscular disease patients will to some extent involve the effects of disuse, if only that *forced* upon the patient by the illness. Support for this is the fact that atrophy is common to both disuse and neuromuscular disease, and that raises the hope that a quantitative impedance study focusing on disuse by itself would shed light on corresponding features commonly encountered in dealing with the diseases.

Part of atrophy's effect on the impedance will certainly be attributable to alterations in the dimensions and layout of the muscle fibres, which we will refer to as "anatomical" changes, but one cannot exclude what we will refer to as "physiological" changes as well, for example the growth of collagen networks (fibrosis) and changes in ionic concentrations of the various compartment fluids. Sorting these out and discerning their relevance to disease will be challenging, and the first goal of the present research is simply to determine whether as a practical matter the new technique is sensitive enough for dealing with disuse following bone fracture alone. The second is to study whether one can distinguish "anatomical" from "physiological" effects under these relatively simple conditions, and thereby to offer clues to the more complex changes occurring in disease.

An overview of the background and methodology is given by figure 1. The main subplots illustrate the magnitudes and character of the effects to be expected, comparing the frequency dependencies of R and X for the two medial gastrocnemius muscles (MG) of a patient who suffered fracture of the right ankle (distal fibula), followed by casting and prohibition of weight bearing. (Of course details vary amongst patients and stages of recovery.) Also, the inset shows the 5-element circuit which lies at the core of the analysis – i.e. finding the set of parameters which best fits the model to these and similar $R(f)$ and $X(f)$ data, and where possible following their changes during recovery. The frequently adopted association of the parameters with the anatomy is that r_1 represents the extracellular fluid, r_2 the intracellular fluid and c_1 the muscle fibre membrane, to which is added that r_3 and c_2 together represent the collective properties of the intracellular organelles and fluids. (A topologically distinct 5-element model has been proposed by Gersing (1998) to explain impedance changes due to closing of gap junctions which may occur in transplant surgery or other long duration ischemia. The $R(f)$ and $X(f)$ functions are identical to those for the version adopted here (an example of the ambiguity discussed by Barsoukov and MacDonald, 2005), though of course the anatomical interpretation of the parameters is very different.)

II. Methods and Subjects

A detailed description of the technique, the apparatus, and the analysis of the impedance data has been given by Shiffman et al. (2008). While that paper dealt with frequencies up to 2 MHz, the work presented here is restricted to measurements in the 3 to 300 kHz range, as

were Part I, dealing with disease, and Part II, dealing with normal subjects. The first of these also demonstrated the superiority of the 5-element model over the often adopted 3-element model, and the second also dealt with questions of reproducibility and consequent parameter error bars, based on test-retest assessment of healthy subjects. These show that experimental uncertainties expected in the present work are $\sigma_{rep} = 6, 6, 16, 11, \text{ and } 12\%$ (rms) for individual extractions of $r_1, r_2, r_3, 1/c_1$, and $1/c_2$, respectively. (Part II also explains why it is appropriate to regard $1/c_1$ and $1/c_2$ rather than c_1 and c_2 as the “capacitance parameters,” and it introduces the term “cellular parameters” to mean the sub-set $r_2, r_3, 1/c_1$ and $1/c_2$.)

In what follows data will be labeled by muscle names such as “biceps”, “tibialis anterior (TA)”, and so forth, but these are simply shorthand for muscle regions, indicating the placement of the voltage measuring electrodes. They should not be taken to mean the technique is capable of actually selecting individual anatomically distinguished muscles. So for example, “quadriceps” designates an array starting 5 cm above the proximal edge of the patella and extending 17.5 cm proximally along a line parallel to the thigh axis, and “forearm” designates a 10 cm long array over the collection of finger flexors on the anterior forearm. (Fig. 3 of Part I shows a sketch of a typical setup, and a complete description of the arrays is given by Esper et al (2006). As before, arrays of voltage electrodes were employed for the purpose of establishing that the data consistently represented the properties of the underlying tissue, but the results reported here always pertain to the maximum span of the array.)

Since the circuit model does not take muscle dimensions into account, the results are couched in terms of the *ratios* of parameters for the injured and contralateral healthy muscle regions for the same subject. This amounts to regarding the un-injured contralateral muscles as individual comparison standards, thereby rendering unnecessary the use of age and muscle-mass adjusted mean values of a normal “control group.” Contralateral muscles may not entirely escape the consequences of the fracture, e.g. as a result of unaccustomed burdens or activities, but the effects are expected to be very small (White et al., 1984). We note also, that the clinical records for all subjects showed no evidence of prior injury on either side. Consequently, the use of injured to contralateral parameter ratios is very much preferred over the “girth-normalization” method required for example in Part I. An expected side benefit is that systemic effects will tend to cancel.

All subjects were patients in the Orthopedics Department of the Beth Israel Deaconess Medical Center of Boston (BIDMC), either on an emergency basis or for follow-up examination. The data presented here were culled from the broader survey described in Parts I and II, and accordingly no selection criteria could be adopted regarding fracture type, duration of the cast immobilization period or the stage of recovery. Eighteen subjects were examined, 10 males, 8 females, aged 18-66 yrs, (mean 38), and informed written consent to the impedance measurements was obtained in each case, in accordance with the policies of BIDMC.

One patient was examined in greater detail than the others and warrants more complete description. He was a 52 year old man whose impedances were first measured within two days of fracture of the distal right fibula. He was placed in a short leg cast and measured again at 27 days, by which time swelling of the ankle area (out of cast) was reduced and the cast replaced. At $t = 41$ days the hard cast was changed to an air cast allowing weight-bearing, with ankle and foot still “somewhat swollen” according to the clinical notes. The final impedance measurements were made 219 days after the injury, at which point recovery was clinically judged complete, though fracture lines were still visible on X-ray and the patient reported mild discomfort on extensive walking.

III. Results

There were 50 instances where a muscle and its contralateral partner were examined on the same day, allowing injured-to-uninjured parameter ratios to be calculated. An illustrative sampling is given in Table 1, together with the mean values and standard deviations for the selected sample and for the full 50 case set. In addition to the parameters, a column has been added showing the (inverses of the) injured-to-healthy 50 kHz phase ratios, such as were measured by Tarulli et al. cited earlier. Missing from the table is any indication of the duration of disuse, since that is virtually impossible to specify. Some subjects were allowed to use the injured limb very early in recovery (“as tolerated” according to the clinical notes), and for others the impedance data were obtained only after all restrictions were removed. However amongst those for whom it was possible to reasonably assign a value, the disuse periods ranged from 28 to 63 days.

The columns for the parameter ratios can be regarded as the equivalent of Table 2 of Part I, in which corresponding results are shown for the various disease groups. For disuse just as for disease the mean values for the ratios of the “cellular” parameter ratios (r_2 , r_3 , $1/c_1$ and $1/c_2$) are clearly very much larger than one ($p < 10^{-8}$), and once again the r_1 -ratio is certainly very close to 1 (i.e. $p = 0.81$.) Note that the standard deviations given in the last two rows of the table represent the spreads amongst the tabulated values not the uncertainties in the means, which will be needed later. Under the usual assumption that the spreads are normally distributed, the standard deviations for the (full group) means are 0.014, **0.006**, 0.042, 0.071, 0.071 and 0.042 for r_1 , r_2 , r_3 , $1/c_1$, $1/c_2$ and $1/\omega$ respectively.

As in the case of neuromuscular disease there was a wide range of correlation coefficients between the different parameter ratios, and again the correlation between the $1/c_1$ and r_2 ratios was the strongest, at 0.85 (somewhat lower than the 0.91 average found for the diseases.) In Part I it was stated that plots of the r_2 vs $1/c_1$ ratios were reasonably good straight lines, (with disease dependent slopes), and that holds for disuse as well. This is demonstrated in fig. (2), from which the least squares fitted slope is 0.55 ± 0.03 . (According to Table 3 of Part I, this is smaller than for any of the tested diseases, which range from 0.62 ± 0.06 to 1.49 ± 0.05 .)

A more striking demonstration of the qualitative agreement between disuse and disease is found by calculating the fractional changes in each parameter, $(p/p)_i = (P_{\text{injured}}/P_{\text{uninjured}} - 1)_i$, and then averaging over all members of the group, i.e. $P(i) = [p_i/p_i]_{\text{grp avg}}$. Figure (3) shows this for the two groups as a pseudo-graph in which the $P(i)$ for the fracture-disuse group have been multiplied by 2. The figure has arranged the parameters in the order of increasing $P(i)$ as found for the disease group, i.e. r_1 , r_3 , $1/c_2$, r_2 , $1/c_1$, (same order for both groups), which has the consequence that the “graph” is roughly linear. That is pure accident, and presenting them in the r_1 , r_2 , r_3 , $1/c_1$, $1/c_2$ order used up to this point would simply lead to two intertwined upper case “M” patterns instead. The important result is that whatever order is used, a *single* multiplier of the five disuse $P(i)$ results brings them into good agreement with those for disease. The order adopted in the figure does have the advantage, however, that it is easy to see relationships between parameter changes, for example that r_3 is about a third as sensitive as $1/c_1$ to whatever it is that causes the impedance to change. Also, while age has substantial influence on the parameters (cf. Part II) the *ratios* and group averaging involved in the definition of P make inconsequential its contributions to the standard deviation “error bars” in the figure.

As stated, the selection of disuse subjects was largely a matter of chance, and in only one case was it possible to follow the healing process from very soon after injury to what was deemed full recovery. A 52 year old male had fractured his right ankle (distal fibula) and

impedance measurements were made on 4 occasions, running from about two days after the accident to clinical discharge 219 days later. Measurements and 5-element analyses were done for the quads, TA's and MG's, and fig.(4) shows the TA results. The first 5 subplots sketch the time dependencies of the parameters r_1 , r_2 , r_3 , $1/c_1$, and $1/c_2$, using solid lines to indicate the non-weight-bearing stage and dashed lines the progressively weight-bearing recovery period, this last in recognition that recovery may well have been much faster than solid lines might imply. Except for r_1 , all the parameter subplots are dominated by major changes in the injured side parameters during the non-weight-bearing period. Also, the post-fracture but pre-casting values for the two legs are within normal left-right asymmetries, i.e. the trauma *per se* had little or no immediate effect on the impedance. There is evidence for a small increase in r_1 for *both* legs over the full 219 days, just exceeding estimated experimental errors, and all four of the "cellular" parameters are still slightly larger on the right side than on the left. However only for r_3 is the final right-left difference verging on significance ($p \approx 0.06$). The sixth subplot shows the average values of the girths measured along the voltage electrode array, in which the range of the y-axis has been restricted so as to emphasize that any differences between the two legs were very small; the mean girth ratio for the 219 day period was 1.01 ± 0.015 .

Large right-left differences during the initial, non-weight-bearing stage were also found for the other tested muscles of this subject, those for the MG even more marked than for the TA and those for the quadriceps less so. As indices characterizing the overall behaviors we have calculated the right-to-left parameter ratios for each visit and taken the extreme values found for the full period, and these are shown in Table 2. There is nothing in this procedure to ensure that the timing of the measurements coincided with the actual extrema, and given the rapidity of changes evident in figure 4 it is therefore almost certain that the ratios in the table *understate* the differences during the fracture/recovery period. But even so the effects of the 41 days of non-weight bearing are dramatic, particularly for the MG. There the cell membrane capacitor c_1 fell by a factor of at least four and the intra-cellular capacitor c_2 by a factor of at least three, but again r_1 appears to be much less sensitive to disuse. As in Table 1, the last column shows the inverses of the 50 kHz phase ratios, which are seen to be as much as a factor of two less sensitive than the capacitive parameters. On the whole the ratios are more extreme than those given in Table 1 (and for the full disuse group), as is to be expected given the "random timing" of the measurements presented there, in particular the resumption of weight bearing for some of its entries.

IV Discussion

A. Comparisons between disuse and neuromuscular disease

Table 1 represents a retrospective survey of single-visit, injured-to-contralateral impedance differences following bone fracture, in which the duration of disuse and the timing of the measurements relative to the fracture were uncontrolled. In that respect it reflects similar limitations in the study of neuromuscular disease (Part I), in that the onset of disease is invariably gradual and not well defined, and data for unaffected as well as affected muscles on the same subject were often unavoidably included. There was also no independent way of objectively characterizing the severity of the various diseases, just as here there is no independent way to quantify the effects of the various fractures. Nevertheless it is instructive to compare overall susceptibilities of the parameters in the two studies, and this is done in Table 3. The first row lists the injured-to-uninjured parameter ratios averaged over the full set of examined muscle pairs, while the second shows the diseased-to-(age-matched) normal parameter ratios from Part I, weighted by the number of subjects in each disease category. (The indicated "errors" are the standard deviations of the means.) That the disease ratios are somewhat larger than those for disuse must be regarded as pure chance, but the much larger SEM's for disease undoubtedly represents the wide range of disease types and severities

which are represented – including muscles not (yet) affected at the time of measurement. In contrast every muscle in the disuse group is associated with fracture.

The table demonstrates that the effects of disuse following (uncomplicated) fractures are comparable to what is found for serious disease, in fact quantitatively the same insofar as r_1 is concerned. Together with the evidence that correlations amongst the parameters are qualitatively the same for disuse as for disease (fig. (2) and Table 3 of Part I), this suggests that a major part of the impedance changes in both categories can be traced to a single underlying cause. A missing and critical element in testing this hypothesis is the lack of any independent way of quantifying the muscle disturbance, whether due to disuse or disease. Specifically, both presentations obscure the fundamental issues of severity and duration and the pragmatic issues of measurement and parameter extraction uncertainties. Part I dealt with the complexities of neuromuscular disease in these contexts, drawing on Part II for test-retest evidence of the reliability of the parameters. But disease progress was so slow (in many subjects the disease was incurable) that the terms severity and duration were qualitatively equivalent. In contrast recovery from bone fracture is virtually assured and consequently so rapid that in making comparisons between individual cases one must be sure to take into account the stage and conditions of recovery under which the measurements were made. Failing that there is no way to explore further differences such as exhibited in Table 1.

But in the case represented by fig.(4) we can at least be roughly certain of the timing of a definable stage of the recovery, between 27 and 41 days, where maxima were observed in all the parameters (except r_1 .) The “extreme” ratios given in Table 2 pertain to that period, and we combine these with the reproducibility standard deviations σ_{rep} from Part II, (and repeated here in section 2) to form the “effective signal-to-noise ratios” or “operational sensitivities” p/p_{rep} shown in Table 4. Two features emerge clearly, first that the fractional changes can be over 20 times the overall system uncertainty, so just as in dealing with disease there is more than enough sensitivity and reliability to recommend the technique for clinical use. Second, the hierarchy of effects amongst the three muscles is intuitively consistent with the location of the fracture, the ankle, at first sight at least. However more than non-weight-bearing or general disuse must be involved, since all three muscles were subject to the same duration and (presumably) the same degree of disuse. The differences between the values labeled “MG” and “TA” are particularly striking, especially those for $1/c_1$, and it certainly appears that the “MG” was very much more seriously affected by the disuse regimen than was the “TA”. We include the inverted commas at this point as a reminder that the designations refer to the locations of the voltage measuring electrodes (cf. section 2) and they must not be taken to imply that the technique is able to discriminate between superficial and deep muscles lying under the electrode array. Certainly both the proximity of a muscle and its volume must affect its influence on the impedance, and with that in mind one must expect that the parameters assigned to the “MG” must reflect properties of the lateral as well as medial gastrocnemii and especially of the soleus. (Even the tibialis anterior and posterior muscles likely contribute, though their volumes are so small by comparison that one expects little effect.) Similar considerations apply to the “TA”, though there the volume factors lead one to expect that the TA itself would play only a minor role. But despite these uncertainties there is one feature that clearly distinguishes the two groups, namely their points of attachment to the foot. That is, all of the “MG’s” attach to the calcaneus via the Achilles tendon, which traverses the site of the fracture (distal fibula), while the “TA’s” attach to the metatarsal bones, skirting the region of the fracture.

The implication here is that the differences in the effects of disuse may reflect the “tendon paths” between the muscle and the site of the injury. Obviously this can only apply to cases where there *was* an injury, and it is known that different muscles of the same (animal)

subject are affected differently in zerogravity, where there is no “injury” at all (Martin et al. 1988). This emphasizes the more general problem noted earlier, that there is as yet no way to independently connect the impedance changes to underlying physiological conditions. So in terms of the MG and TA comparison just discussed, we cannot rule out that, for example, the physiological changes in $1/c_1$ were the *same* for the both, but that the MG is simply 3 times *more sensitive* than the TA. Or in terms of the broader goals of this paper, how can we be sure that a given parameter ratio means the same in *physiological terms* for a neuromuscular disease patient as for a fracture/disuse patient?

There are *inter-parameter* relationships which demonstrate similarities between disuse and disease even in face of that lack. The insensitivity of r_1 to both disuse and disease and the linear correlation between r_2 and $1/c_2$ have already been noted. But probably the most important is the one represented by fig.(3). This displays average fractional changes in the parameters for the two groups, and it strongly suggests that there is an underlying pattern of changes which is the same for disuse following bone fracture as for neuromuscular diseases (at least for those examined in Part I.) The most straightforward explanation for fig.(3) is that for each parameter “p” the fractional change $\Delta p/p$ for an individual subject is the product of a *severity*-independent parameter factor and a *parameter*-independent severity factor. This would be expressed mathematically by $P_k(i) = S_k f(i)$, where S_k is the severity factor for patient “k”, and $f(i)$ specifies the relative response of the i^{th} parameter p_i to an underlying common mechanism. (The actual form of f will depend on the order in which the parameters are arranged. For the order used in the fig. (3), $i = 1$ designates r_1 , $i = 2$ designates r_3 , and so forth as indicated along the abscissa.) Of course S_k will vary from subject to subject, which muscle is being considered, whether affected by disease or disuse, mild or severe and so forth. Furthermore just how one collects subjects into groups is arbitrary. Nevertheless averaging over all subjects within each group would give a result of the character depicted in fig. (3), i.e.

$$P(i) = S \cdot f(i) \quad 1$$

in which the absence of the subscript “k” indicates that $P(i)$ and S are now group averaged values. The actual value of S (and the sizes of the “error” bars in the figure) would of course depend on just how the groups were chosen; for example S would be larger for a group involving patients with very serious disease (or disuse) than for a group with only marginally affected subjects. Also, since there is no way to define severity, one can take it to be unity for one group and ask if there is a value for the other which leads to confluence of the two versions of eq.(1). In fig. (3) we have set $S = 1$ for the disease group, and it is an accident of the populations of the two groups that choosing 2 for the disuse group leads to the overlay shown in the figure. The point is that there *is* such a factor. (Restricting the disuse group to the “specimens” in Table 1 leads to even better overlay, but with $S = 1.5$.)

Many different diseases were included in the disease group, and there were differences *within* it as large as the differences between the averages for the two groups. Taken together with the fact that the inter-parameter pattern of group averages is the same, this reinforces the hypothesis that disuse and neuromuscular disease share a common mechanism for impedance changes. It must be emphasized that the term “common mechanism” as used here refers to a mechanism *for impedance change*, certainly not for the different diseases or injuries. An analogy might be that a substance “M” is generated by any of a number of physiological disturbances and it is simply the amount of “M” that determines the impedance changes. If one changes the word “substance” to “condition” one has a more plausible version: the existence of a common pattern of parameter changes argues that there is a particular physiological condition which develops in both disuse and disease, and the pattern of changes amongst the parameters is characteristic of that condition.

Fig.(3) deals with group-mean values and the “error bars” shown there more than likely reflect genuine differences amongst group members than measurement errors. So whatever the common mechanism might be there is no reason to exclude other factors influencing individual cases. In particular one must allow that there may be entries in major conflict with the pattern shown there, but whose egregious contributions have been muffled in the averaging. Prominent examples are some of the most seriously diseased cases of Part I, e.g. the unique behaviour of r_3 in inclusion body myositis (IBM), the breakdown of the r_2 - $1/c_1$ correlation in severe amyotrophic lateral sclerosis (ALS) and the factor of 20 found for the quads r_2 ratio in a case of Charcot-Marie-Tooth disease (CMT). The data of the present Table 2 also offer an example, now involving disuse rather than disease. According to the table, the right-left parameter ratios for $1/c_1$ and $1/c_2$ for the MG and TA were the most seriously affected, but plotting and scaling the (p/p) as in fig.(3) shows that it is r_2 which is the “bad actor”; the 13 other (p/p) values fit into the overall pattern quite well. Put another way, all five parameters for the quads follow the pattern of fig. (3), and so do those for the MG and TA *except* for their r_2/r_1 values, which fall well below the range shown there.

B. Mechanisms for the parameter patterns

In the search for the putative common mechanism one must certainly include atrophy and fibrosis, since both are known to occur in disease and disuse. If only in dealing with these, the search will require investigations well beyond the reach of impedance studies alone, but some preliminary steps can be taken using the literature for extended disuse following fracture. Before doing so it is well to establish two requirements mandated by the gross features of fig. (3): first the mechanism must leave r_1 virtually untouched, and second it must be compatible with the fact that r_3 is much less strongly affected than are the other three cellular parameters $1/c_2$, r_2 , and $1/c_1$.

Starting with atrophy, we note that in virtually every case examined here the left-right differences in limb *girths* were very small, and that is certainly so for the subject of fig.(4) (cf. subplot 6.) But as Sargeant et al. (1977) have demonstrated, such “anthropometric” techniques can substantially underestimate the actual degree of muscle fibre shrinkage -- by as much as a factor of 3 in their work. One must therefore use only direct assessments of fibre atrophy, and fortunately there are such studies dealing with injuries and disuse durations very similar to those encountered here. In particular, Psatha et al. (2012) found cross-sectional areas of lower leg-muscle fibres falling by up to 20% in 43 days of cast immobilization following ankle fracture, using MRI, and Sargeant et al. found area shrinkage averaging 42% (for the vastus lateralis) for tibia and fibula fractures immobilized for an average of 130 days, using biopsies. Since it is highly unlikely that the fracture patients examined in the present work were in any way exceptional, we should expect that shrinkages of comparable magnitude occurred here as well, and in fact the Psatha conditions are almost exactly the same as those for the subject of fig.(4). But to be more flexible in estimating likely parameter changes we will assume a range of 20 to 40%. Connecting these to the impedance requires a “pseudo-anatomy” and we adopt the model shown fig. (5). Each muscle fascicle is viewed as a collection of regions of cross-sectional area A_0 and length L (into the page), within which there is a fibre in the form of a cylinder of diameter “d”, cross-sectional area A_F and membrane thickness “t.” The space $A_0 - A_F$ represents the endomysium, and the extracellular components of the vascular system are represented by the small filled circles. In this picture “simple atrophy” is defined as shrinking of the fibre diameter from d to d' and we apply this first to the expectations for the parameter $1/c_1$. The membrane capacitance is proportional to Ld/t , so the $1/c_1$ ratio is simply $1/d'$, or 1.1 for the Psatha shrinkage and 1.25 for the Sargeant value. In contrast the observed $1/c_1$ ratios were 2.8 for the TA and 4.2 for the MG. Furthermore, the only way simple atrophy could influence the *intracellular* parameters would be by shrinking of all the internal structures, but

we see either from Table 2 or fig.(3) that no matter what scaling rule was applied the behavior of r_3 could not be explained. Variants of this simple picture have also been examined, for example that “ t ” changes (very unlikely, given its bilipid structure) or that the perimeter does not shrink in keeping with the reduced cross-sectional area, and hence the membrane corrugates to compensate (cf. Rothschild, 1957). But these make matters worse.

“Simple atrophy” is then virtually ruled out, but it is a flawed construct to start with. Indeed, the contrast between anthropometric and direct measures of fibre shrinkage indicates that much of the volume of the muscle cannot be accounted for. In neuromuscular disease that is often compensated by increases in intramuscular adipose tissue (see e.g. Mora, 1989), and shrinkage with disuse is usually accompanied by fibrosis, in particular the growth of collagen connective tissue in the endomysium (Williams and Goldspink, 1984, Gillies and Lieber, 2011). Collagen consists of long, very thin fibres, and its effect on electrical properties will depend critically on whether they occur in sparse networks or in tightly bunched compacts. Distributions of both kinds have been reported by Jarvinen et al. (2002) and Gillies and Lieber (2011), in particular that dense accumulations may form directly on the fibre membrane. It is not possible to say whether these are permeated by the extracellular fluid, but even if only the part closest to the sarcolemma is impermeable then the effect would be to increase the effective value of t , the membrane thickness. That is so small (e.g. 20-30 nm) that the impact on c_1 could be very large. For example if we assume that the cross sectional area of an added compact collagen layer was 1% of the fibre area and take $d \approx 50 \mu\text{m}$ and t 25 nm then the $1/c_1$ ratio turns out to be about 5 ! Given the various assumptions one cannot take that result seriously, but it does illustrate that the fibrosis hypothesis might offer an explanation for the substantial increases in the $1/c_1$ ratios. On the other hand, the presumed collagen system lies entirely outside the fibre, and it is very difficult to see how it could explain the large changes found for r_2 and c_2 .

Similar arguments can also be applied to the behaviour of r_1 , at least in its customary interpretation. If d simply shrunk to d with no change in the resistivity of the extracellular fluid, then r_1 would be inversely proportional to $(A_o - A_F)$, which increases as d decreases. But if A_F decreased by 20- 40%, as indicated above, the small changes which are found for r_1 could only mean that A_F was originally a small fraction of A_o . Numerical estimates of this effect can also easily be made. To reconcile the r_1 -ratios of Tables 1 and 2 with the Sargeant et al and Psatha et al shrinkages requires that in the *healthy* muscle the fascicles were 55 to 75 percent endomysial tissue, far above what is known from direct anatomical studies. To be sure one should also consider that the *resistivity* of the extracellular fluid changes under atrophy, and in fact Ishikawa et al. (2005) report an almost 30% drop in hyaluronic acid concentration in the muscle of hind-limb suspended rats. However it is easy to show that it would require a *doubling* of the resistivity to bring the endomysium- to-fascicle area ratio down to a much more acceptable value, say 20% . There is no assurance that the same effect occurs in humans, and furthermore no way to quantify the effect on the impedance without knowing the overall ionic concentration, but the required 100% change in resistivity must be regarded as extremely unlikely.

One cannot rule out that particular combinations and variations of these simple atrophy/fibrosis models might be contrived to account for the behavior of r_1 but a far simpler course would be to adopt the proposition advanced in Part I, that r_1 simply is not a property of the muscle *per se*. The re-interpretation was suggested as a way of explaining how r_1 managed to escape the ravages of a wide range of neuromuscular diseases, and it was supported by the findings in part II that it was virtually insensitive to normal aging as well. The fracture/disuse data are certainly compatible with the new interpretation, but more to the point of the present section there is no need to search for some feature of *muscle* anatomy or physiology which can explain it.

Part I went beyond disassociating r_1 from the total extracellular fluid of the muscle by suggesting that it should be assigned to the vascular component alone. Interestingly, the present study gives independent evidence for a “physiological” change affecting r_1 which is qualitatively consistent with this re-interpretation. The work of Ishikawa et al. cited earlier showed that the decrease in hyaluronic acid concentration in the interstitial fluid during atrophy was accompanied by a corresponding *increase* in the concentration in the vascular fluid. So if r_1 is associated with the vascular component of the extracellular fluid then it would be expected to drop during atrophy, though once again there is no way to estimate by how much. Nevertheless it is interesting to observe that when the early ratios for the right TA are examined closely (cf. fig. 4) one does find that r_1 did decrease initially. The rate is only $0.08 \pm 0.003 \text{ days}^{-1}$ ($R = 0.97$), but at 43 days that amounts to about twice the standard deviation for the distribution of r_1 ratios. (The initial slopes for the MG and quads are approximately -0.009 and -0.003 , resp.) While one cannot rule out that this is just a statistical aberration, it is also possible that it represents a real early-stage effect, common to all the cases we examined but obscured by the random timing of those measurements. Correspondingly such early drops in the r_1 ratio may well support the reinterpretation of r_1 as the vascular component of the extracellular fluid.

(One should allow that part of the extracellular current may flow parallel to but outside the vascular system, still entirely avoiding cell membranes. Accordingly the claim should not be that r_1 represents *only* the vascular current but rather that it is dominated by currents flowing through the vascular system. Also, it might be objected that the much larger interstitial component of the extracellular fluid is ignored under this hypothesis, but that is not the case. As noted in Part II, the interstitial current spreads along the very long surface of the fibre membrane and couples to its interior through c_1 , where it contributes to the intracellular current.)

C. The time dependence of the parameter changes

The one case where even a rough time dependence was available supports the view that the impedance changes are compatible with established clinical stages of recovery. First, it is clear from fig. (4) that the time scale for initial major changes is 30 to 50 days. (The figure includes results only for the TA's, but the same holds for the MG's and Quads.) Such a time span is well in keeping with what is observed in orthopedic and other assessments of disuse following fracture, and White et al. (1984) report even faster changes in voluntary cast-immobilization experiments. Second, while circumstances disallowed further examinations until that patient returned for a final check-up (about 5 months later), both the clinical and the impedance assessments indicated complete or nearly complete recovery at that time. Correspondingly, it is reasonable to assume that the overall pattern of parameter changes in fig.(4) qualitatively mirrors clinical descriptions of disuse and recovery, and in particular that the extreme ratios found for the initial period are highly likely to be the extreme ratios for the entire 219 day span. With this last and the relevant clinical histories in mind, the ratios in Table 1 are certainly compatible with the patterns sketched in figure 4.

An important practical result is that the rates of change of the cellular parameters can be very large compared to what is found in the literature using other techniques. For example, the maximum in the $1/c_1$ ratio for the MG of Table 2 was reached in 27 days of immobilization, which corresponds to a rate of change of almost 12% per day. Rates between 2 and 6% per day are found for the other “cellular” parameters in fig.(4), and as explained earlier these are almost certain to be underestimates of the true initial rates. In contrast, the MRI results of Psatha et al. for nominally the same fracture and disuse time translate into rates from only 0.3 to 0.6 percent per day. Weightlessness is also known to induce muscular degeneration (Vandenburgh et al. 1999), but since the measuring systems

needed for direct measurements on astronauts cannot be carried aloft the available data can only represent conditions before launch and on return to earth. For example the space-flight data of Akima et al. (2000) showed that knee extensors and flexors lost cross-sectional area at an average rate of 0.8% per day, again according to MRI, and Qin (2010) reports decreases in the principal leg muscle volumes which translate into rates of only about 0.1% per day for a 6-month space mission. Taken at face value, these make sense only if the effects of microgravity saturate and one were simply dividing much the same numerator by considerably larger denominators, though of course other factors may have played roles as well. But the comparison emphasizes the advantage that *in-flight* assessments would have for monitoring muscle condition during extended orbital stays and during the long interplanetary journeys being planned. The present work testifies that the MFEIM/5-element analysis qualifies as a useful and easily implemented “air-borne” tool.

V. Summary

Two questions were posed in the introduction. The first was whether the parameter-extracting multifrequency technique was adequately sensitive to disuse, and the answer is definitely “yes,” particularly if measurements can be made in the early stages of immobilization. Indeed, disuse following ordinary, complication-free fractures can have effects comparable to those due to very serious neuromuscular disease. Furthermore, since the loss of muscle mass and strength in microgravity is similar to what occurs with lack of weight-bearing in fracture/disuse, the technique offers a potentially important tool for use in space flight. Compared to other techniques its simplicity, light weight, sensitivity and speed make it particularly appropriate for in-flight assessments of muscle conditions and the development of countermeasures.

The second question was whether the study of disuse would benefit the interpretation of MFEIM as applied to disease, and there we believe the answer is yes on two counts. First r_1 is found to be almost totally insensitive to disuse just as it is largely immune to disease and normal aging. In that way the results support the hypothesis advanced in Part I that r_1 should be regarded as characterizing the vascular rather than the total or the interstitial component of the extracellular fluid. Second, when the results for neuromuscular disease and disuse are combined one finds that the *pattern* of changes amongst the parameters of the circuit model is fundamentally the same regardless of the category or severity of the disorder. This strongly suggests that there is a common physiological condition dominating the generation of the impedance changes, and that what distinguishes individual cases is the extent to which the relevant disease or injury engenders that condition.

An effort was made to identify the putative “common mechanism” in terms of atrophy and fibrosis, common to both disuse and muscular disease, and that required that values of fibre shrinkage and the like be taken from MRI and biopsy results in the literature. Although cases were found which closely mimicked those for which impedances had been measured, the attempt was unsuccessful. But it served to emphasize that to make progress one will likely need impedance, imaging and physiological data for the same subjects. A combined study would be most useful in resolving doubts or in altering the “pseudoanatomy” on which the search for correlations between impedance and anatomy/physiology has been based. Once accomplished then the interpretive quest could turn to the fundamentally more interesting questions of what changes in the other cellular parameters may signify, i.e. r_2 , r_3 and c_2 , both in disuse and disease.

Acknowledgments

We are pleased to acknowledge helpful and stimulating discussions with Prof. R. Aaron, and S.R. Rutkove, MD, and the assistance of Mark Martino, Phillip Mongiovi, Patricia Michelle Fogerson and Nicholas O'Connor in acquiring and processing much of the impedance data.

This research was supported in part by the U.S. National Institutes of Health, under grant number RO1-NS04203

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Figure(1).

Frequency dependences of (a) resistance, R , and (b) reactance, X , for the right and left MG's of a 52 year old male subject who suffered fracture of the distal right fibula roughly 40 days before the impedance measurements shown here. All evidence points to the subject being otherwise quite normal; for example the 50 kHz phase on the left MG was 10.4° , well within the range found for healthy subjects of the same age and sex in Part II (and virtually constant throughout the recovery period.) The inset in (a) shows the 5-element equivalent circuit used in the analysis of these and similar data.

Figure 2.
Correlation between the $1/c_1$ and r_2 ratios

Figure 3.

Common pattern of group-average changes $P(i) = \Delta p_i / p_i$, here p_i is the i -th parameter in the order shown along the abscissa. Squares: 2 times the $P(i)$ for the fracture/disuse subjects of this study; circles the $P(i)$ for the neuromuscular disease patients of Part I

Figure 4. Time dependence of the circuit parameters for the left and right TA's of the 52 year old man during recovery from fracture of the distal right fibula. Triangles are for the right (injured) leg and circles for the left (uninjured) leg. The 6th subplot illustrates the constancy of the respective girths.

Figure 5.

Cross-section of the “pseudo-anatomy” model. The muscle fascicle is represented as a set of space-filling regions of typical area A_0 and length L (into the page) each containing a fibre of area A_F , diameter d and membrane thickness t . The cross-sectional area of the extracellular space is proportional to $A_0 - A_F$, exaggerated here for clarity. Solid circles represent the extracellular vascular system.

Table 1

Specimen “randomly timed” injured-to-contralateral-side parameter ratios

fracture	muscle	r_1	r_2	r_3	$1/c_1$	$1/c_2$	$1/_{50}$
41 y.o.f. radius FX	Forearm	1.2	1.6	1.4	2.3	1.7	1.3
	Biceps	1.0	1.2	1.1	1.3	1.4	1.3
52 y.o.f. radius FX	Biceps	1.0	1.3	0.7	1.9	1.5	1.3
	Forearm	1.1	2.0	1.3	2.7	1.5	1.8
31 y.o.m. malleolus FX	Quads	1.03	2.2	2.0	1.7	1.1	1.6
	TA	1.04	1.9	2.1	2.4	2.1	1.5
26 y.o.m. fibula FX	MG	0.8	1.7	1.5	2.7	2.4	2.0
	TA	0.9	1.3	1.3	1.8	1.7	1.4
	Quads	1.01	1.3	1.4	1.2	1.2	1.2
66 y.o.f. wrist FX	Forearm	0.95	2.0	0.7	3.2	1.3	3.7
	Biceps	1.1	1.4	0.9	1.5	1.2	1.4
50 y.o.f. wrist FX	Biceps	1.05	1.4	0.96	1.4	1.2	1.3
	Forearm	1.03	1.2	1.7	1.6	1.1	1.2
57 y.o.m. patella removal	Quads	1.3	2.0	1.4	1.9	1.5	1.2
	TA	0.9	1.1	1.3	1.0	1.4	1.2
	MG	0.8	0.9	1.3	0.9	1.3	1.1
33 y.o.m. patella FX	Quads	0.98	2.8	1.9	2.8	3.4	2.0
	TA	0.96	1.3	1.1	1.4	0.91	1.4
	MG	1.0	1.3	1.3	1.3	1.1	1.4
	r_1	r_2	r_3	$1/c_1$	$1/c_2$		$1/_{50}$
Mean $\pm$ SD for Table:	1.0 $\pm$ 0.1	1.6 $\pm$ 0.5	1.3 $\pm$ 0.4	1.8 $\pm$ 0.6	1.5 $\pm$ 0.8		1.5 $\pm$ 0.6
For full muscle group:	0.95 $\pm$ 0.1	1.4 $\pm$ 0.4	1.2 $\pm$ 0.3	1.6 $\pm$ 0.5	1.4 $\pm$ 0.5		1.3 $\pm$ 0.3

Table 2

52 year old fibular fracture subject Extreme right-to-left ratios for the recovery period.

Muscle	r_1	r_2	r_3	$1/c_1$	$1/c_2$	$1/_{50}$
Mg	0.8	2.1	1.6	4.2	3.3	1.95
TA	0.8	1.8	2.0	2.8	2.4	1.98
Quads	0.9	1.6	1.3	1.8	1.8	1.51

Table 3

Overall mean ratios and SEM's for disuse and neuromuscular disease

	r_1	r_2	r_3	$1/c_1$	$1/c_2$
Disuse (this work)	0.95±.01	1.4±.06	1.2±.04	1.6±.07	1.4±.07
Disease (from Part I)	1.03±0.1	2.01±0.6	1.35±0.3	2.03±0.6	1.67±0.5

Table 4“Operational Sensitivities” p/p_{rep}

Muscle	r_2	r_3	$1/c_1$	$1/c_2$
MG	15.7	7.3	22.9	12.8
TA	8.9	5.3	7.5	6.4
Quads	3.8	0.8	3.0	2.8