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Multi-frequency bioimpedance in equine muscle assessment

Adrian Paul Harrison[1,4], **Vibeke Sødring Elbrønd**[1], **Kiwa Riis-Olesen**[2] and **Else Marie Bartels**[3]

[1] Department of Veterinary Clinical and Animal Sciences, Faculty of Health & Medical Sciences, Copenhagen University, Frederiksberg, Denmark

[2] Hillerød Hestedyrlæger, Baunevej 17, Bendstrup, 3400 Hillerød, Denmark

[3] The Parker Institute, Department of Rheumatology, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Denmark

E-mail: adh@sund.ku.dk

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Abstract

Multi-frequency BIA (mfBIA) equipment has been shown to be a non-invasive and reliable method to assess a muscle as a whole or at fibre level. In the equine world this may be the future method of assessment of training condition or of muscle injury. The aim of this study was to test if mfBIA reliably can be used to assess the condition of a horse's muscles in connection with health assessment, injury and both training and re-training.

mfBIA measurements was carried out on 10 'hobby' horses and 5 selected cases with known anamnesis. Impedance, resistance, reactance, phase angle, centre frequency, membrane capacitance and both extracellular and intracellular resistance were measured. Platinum electrodes in connection with a conductance paste were used to accommodate the typical BIA frequencies and to facilitate accurate measurements. Use of mfBIA data to look into the effects of myofascial release treatment was also demonstrated.

Our findings indicate that mfBIA provides a non-invasive, easily measurable and very precise assessment of the state of muscles in horses. This study also shows the potential of mfBIA as a diagnostic tool as well as a tool to monitor effects of treatment e.g. myofascial release therapy and metabolic diseases, respectively.

Keywords: skeletal muscle, biomedical technology assessment, bioimpedance, myofascial release, BIA, equine, multi-frequency

(Some figures may appear in colour only in the online journal)

[4] Author to whom any correspondence should be addressed. IKVH, Faculty of Health and Medical Sciences, Dyrlægevej 100, 1870 Frederiksberg C, Denmark

1. Introduction

1.1. Background

Bioimpedance has been applied in the medical clinic and in the sports field for assessment of human muscles (Salinari *et al* 2002, Stahn *et al* 2008, Carter *et al* 2009, Rebeyrol 2010, Kim and Kim 2013, Nescolarde *et al* 2013a and 2013b). The method has the advantage of being non-invasive and easily transportable to any setup or outdoor situation. Multi-frequency BIA (mfBIA) has now extended the possible use of BIA to include a much more in depth analysis of the muscle state in terms of training state, exhaustion, injury and metabolic state (Stahn *et al* 2008, Nescolarde *et al* 2013a) and mfBIA has recently shown useful in diagnosing muscle damage and atrophy in a horse (Riis *et al* 2013).

To address as many aspects as currently possible with this method, we have carried out multi-frequency bioimpedance analysis both on a group of healthy horses and on a group of selected cases, that serve to illustrate the potential of mfBIA over more complex diagnostic techniques.

1.2. Aims

The aim of this study was to show the full scale of the application of the mfBIA technique in the veterinary clinic, where we believe this technique could be as useful and powerful a tool in terms of diagnosing and training equine subjects, as has been seen in the applications of mfBIA for human subjects.

2. Materials and methods

2.1. Subjects

Multi-frequency BIA is a safe and non-invasive technique which is easily applied in most settings (Ivorra 2003). All horse owners were informed about the technique prior to giving their informed consent for mfBIA measurements of their horse.

2.1.1. Description of participating horses. Group of healthy horses. A group of 10 healthy and relatively young (6–9 years of age) 'hobby' horses were recruited from the greater Copenhagen area. The average weight was 497 ± 39 kg and the average age was 7.5 ± 0.4 years. Seven male and 3 female horses were used.

Selected cases from the veterinary clinic. Four of these selected patients came from the Equine Veterinary Clinic at Hillerød, Denmark and one came with its owner from the Copenhagen area.

Horse #1: The subject was a 22 year old female riding horse (previously used as a competition racing horse) showing signs of cardio-vascular failure and respiratory distress. The horse presented with regions of tense and contracted muscles, which were following treated by a qualified Veterinarian with myofascial release (Barnes 1990, Ward 2003). mfBIA Recordings of a number of muscle groups on both sides of this horse were taken by an operator blinded to the horse's muscular problems prior to treatment, 1 h post treatment and 24 h post treatment to validate reputed effects of this treatment.

Horse #2: The subject was a 6 year old male horse trained for cross-country carriage competition. This subject was a relatively young and inexperienced horse, keen to perform.

In combination with an older and more experienced horse it participated in a couple of hours of horse and carriage training, with the result that it over-trained. This horse was chosen to illustrate the effects of over-training on involved muscles.

Horse #3: The subject was a 7 year old thoroughbred female riding horse suffering from kissing spine. This horse presented with advanced 'kissing spine'—that is to say touching spinous processes in the thoracic vertebrae (T15–T17). This affects the ability to move normally, to be ridden with a saddle and rider and results in considerable discomfort and pain. Avoidance manoevers to reduce back pain include non-symmetrical contractions of back muscles in an attempt to stabilize the affected region of the back. The horse was chosen to follow post-surgery recovery compared to pre-surgery baseline recordings.

Horse #4: The subject was a 7 year old female competition riding horse with a left-hind limb injury. This horse was chosen as an example of retraining following a muscle injury.

Horse #5: The subject was a 14 year old male Icelandic horse suffering from muscle atrophy and extreme fatigue for a number of months. A blood analysis revealed a low plasma iodine level. Thyroid hormones were in the normal range, although at the low end of the scale, indicating a tendency towards subclinical hypothroidism. This case was chosen to illustrate the use of mfBIA in the assessment of the effects of metabolism on muscle function.

2.2. Bioimpedance analysis

The horses were restrained calmly in a standing position whilst being kept free of all metal surfaces. Precisely determined anatomical areas, which had been previously tested on a number of race horses and other well trained horses to provide control data, were measured (Riis 2013). A conductive paste was applied (Ten20; D O Weaver and Co, Aurora, CO 80011, USA), allowed to warm up to body temperature over a period of 10 min, after which four custom made pure platinum electrodes (10 × 25 mm) were placed on to the prepared sites. The current electrodes were the outer electrodes and the distance to the nearest voltage electrode was always 1 cm, according to manufactorers recommendations. The precise electrode positions are shown in figure 1.

A mfBIA unit (ImpediVET BIS 1, Pinkenba, AU) providing a current of 1000 μA ac was subsequently attached to the electrodes and recordings were carried out six times with a 1 s interval at 256 frequencies over a range of 4 to 1000 kHz. With this approach, any slight movement artifacts or changes in the resistance and reactance values due to cable movement, change in the stance, body, or electrode movement will be minimized.

2.3. Data analysis

The mfBIA data was analyzed using the impediVET software. At the time of recording, the Cole–Cole plot was assessed for a normal distribution and the R and Xc plots were also examined to ensure a precise recording (see figure 2). Following the validation of the recording, a detailed analysis was performed at 50 kHz, where the standard parameters of Z (derived; Impedance (Z) = Square Root ((R Resistance^2) + (Xc Reactance^2))), R (measured) and Xc (measured) were obtained. The Phase Angle (PA) was calculated (arctan Xc/R) and the fc (measured), Re (measured; equal to Ro from the Cole–Cole plot). Ri is then derived using both the measured Ro and R infinity values ($R\infty$; the value at the end of the Cole–Cole plot) from the Cole–Cole plot using the following formula: $Ri = (Re \times R\infty) / (Re - R\infty)$. Mc (derived) is finally calculated from the measured fc (the frequency that gives the maximum Xc value on

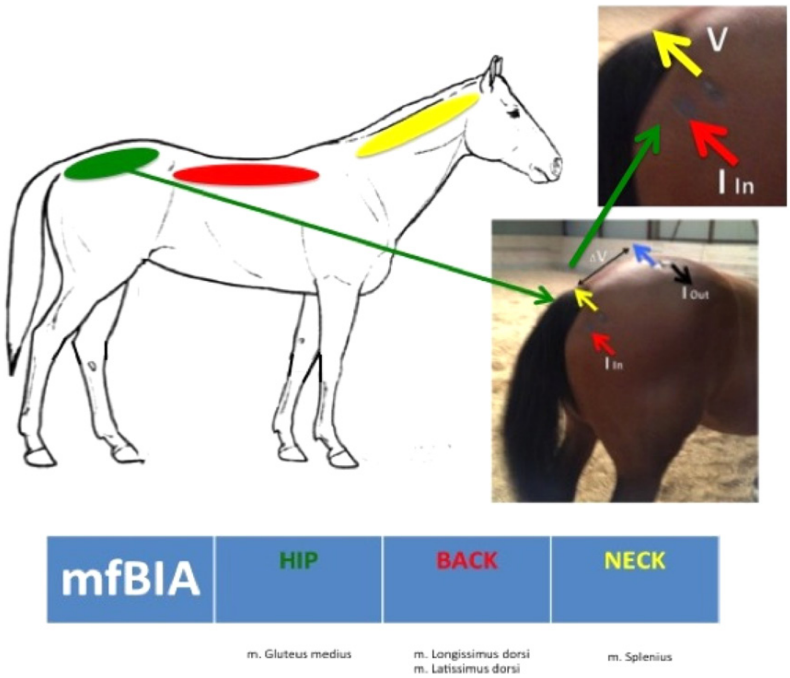

Figure 1. The muscle regions used for measurement are identified by their colour and their muscle name. The lower right-hand insert shows a photo of a typical electrode setup recommended by the manufacturer and adopted in this study for *m.Gluteus medius*. The upper right-hand insert shows a close-up of the electrode placement, which is typically approx. 4 cm between the Current and Voltage electrodes.

ther Cole–Cole plot) and *R*e values and the calculated *Ri* value, using the following formula: $fc = (1 / (2\pi \times Mc \times (Re + Ri))$.

3. Results

3.1. Bioimpedance analysis

A typical Cole–Cole plot from a healthy horse showing signal values in the normal range is seen (figure 2). This plot is, when the recordings are taken correctly, a semi-circular graphical representation of frequency-dependent complex dielectric functions such as impedance, reactance and resistance for the 256 frequencies used. When this plot and the plots for *R* and *Xc* are also acceptable, one can carry out a detailed analysis of all the mfBIA parameters. In order to validate the mfBIA unit used, we spent some time taking repeat measurements using the same electrodes and the same conditions (relaxed muscle; regions; day; horse). We found that measurements taken over a number of hours without any physical activity, resulted in recordings that were identical for all the parameters measured.

3.2. Healthy horses

Values for all mfBIA parameters measured for three muscle groups representing the upper functional line (see figure 1), are given in table 1.

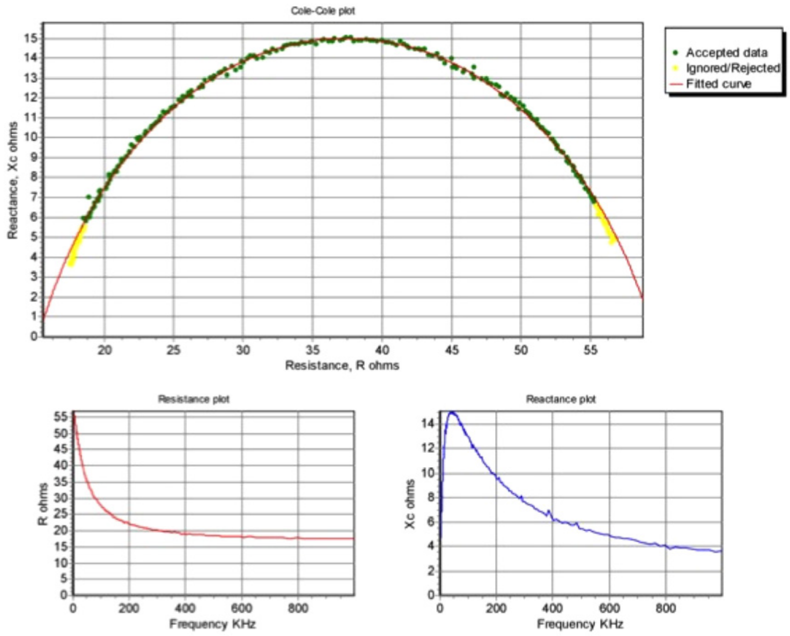

Figure 2. A typical plot of the mfBIA data obtained for a horse. Note the large panel shows a plot of *Xc* against *R* (Cole–Cole plot), whilst the *R* versus frequency and *Xc versus* frequency plots are shown below. These recordings are displayed on the ImpediVET unit at the time of measurement, enabling a real-time assessment of each muscle group. *Re* is equal to the *R* value at zero (the start of the Cole–Cole plot: and a measured value).

3.3. mfBIA case analyses

Horse #1: mfBIA measurements were taken over seven regions/muscle groups prior to myofascial release treatment and then subsequently at 1 and 24 h post treatment. The regions include the three presented in figure 1, as well as the *m. Cranial oblique*, *m. Brachiocephalicus*, *m. Internal Abdominal oblique* and *m. Semitendinosus*. mfBIA values (*Z*, *R*, *Xc* and PA; see table 2) are presented for the *m. Internal abdominal oblique* only and showed no change with myofascial release treatment.

However, a clear difference in terms of the fc value for the *m. Internal abdominal oblique* muscle (see figure 3) was observed, while the other muscle regions did not show any changes. Subsequent information from the Veterinarian revealed that this was indeed the muscle region that had been treated. In this case, the effects of myofascial release on the treated muscle had no effect on *Z*, *R* or *Xc*, indicating that this form of treatment did not affect muscle mass or cellular integrity. However, a closer look at the BIA parameters measured over the β-dispersion range (10 kHz–10 MHz) and in particular the centre frequency (*fc*) parameter, did reveal an effect of myofascial release treatment. It was noted that the fc at rest prior to treatment was 60.9 kHz and that this value fell to 51.6 kHz an hour after treatment, falling again to 46.8 kHz at 24 h post treatment. This form of measurement in connection with myofascial release was repeated on another horse, with similar results (values not shown).

Horse #2: The *m. Gluteus medius* was measured as shown in figure 1. The normal resting values for both *Xc* and PA for *m. Gluteus medius* were measured to be 23.3 Ω and 22.3°,

Table 1. Hobby horses—mfBioimpedance values for the neck muscle *m. Splenius,* back muscle *m. Longissimus dorsi* and hip muscle *m. Gluteus medius* representing the upper functional line of the horse. Data are the mean ± SEM $n = 10$ horses aged aged 6–9 years, mean body weight 496 ± 39 kg.

	NECK	BACK	HIP
BIA	*m. Splenius*	*m. Longissimus dorsi*	*m. Gluteus medius*
Z (Ω)	63.8 ± 4.9	46.7 ± 2.3	45.9 ± 1.3
R (Ω)	62.4 ± 4.6	45.0 ± 2.2	41.8 ± 1.2
Xc (Ω)	13.2 ± 1.7	12.5 ± 0.9	19.0 ± 0.9
PA (°)	11.7 ± 0.7	15.6 ± 0.8	24.5 ± 0.8
fc (kHz)	42.9 ± 1.9	37.1 ± 0.9	34.5 ± 1.3
Re (Ω)	87.0 ± 8.5	68.2 ± 3.6	76.3 ± 3.0
Mc (pF)	22.6 ± 1.2	38.0 ± 3.2	46.2 ± 1.4
Ri (Ω)	82.9 ± 7.9	48.5 ± 4.1	25.3 ± 2.2

Table 2. Horse #1—mfBioimpedance values prior to myofascial release treatment of the *m. Internal abdominal oblique* (PRE) as well as one hour and twenty four hours after treatment (POST + 1) and (POST + 24), respectively.

BIA	Pre	Post + 1 h	Post + 24 h
Z (Ω)	68.10	66.80	66.60
R (Ω)	67.80	66.50	66.20
Xc (Ω)	6.30	6.50	6.70
PA (°)	5.31	5.58	5.78

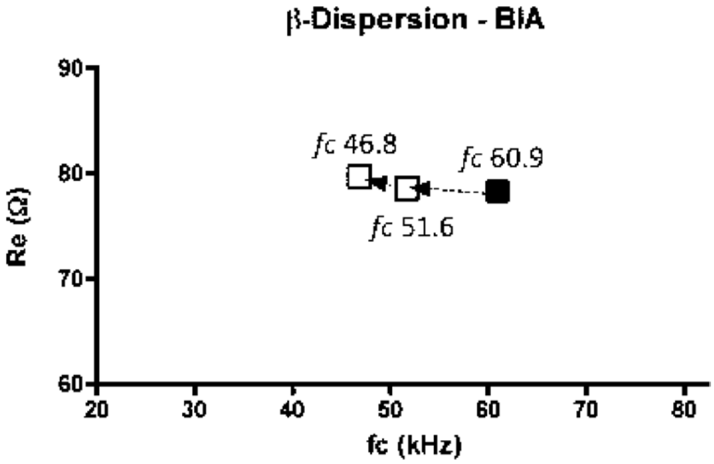

Figure 3. The recorded mfBIA analysis for Horse #1—left to right, prior to treatment (solid square) and 1 h post and 24 h post myofascial treatment. Note the beta-dispersion shows a change towards a lower fc (kHz) with myofascial release treatment, an immediate large response was clearly visible (60.9 to 51.6 kHz), but a further smaller change was also found twenty four hours later (51.6 to 46.8 kHz).

respectively (table 3). After a very strenuous training session, the Xc was approx. 22% of its resting level at 12 h, 75% of its resting level at 36 h and approx. 85% of its resting level at 60 h post training. Similar post exercise changes were seen for the phase angle. It is also interesting

Table 3. Horse #2—mfBioimpedance values pre, as well as 12 h post training and subsequently at 24 h after the initial measurement (POST + 36) and 48 h after the initial measurement (Post + 60) hours, respectively, for *m. Gluteus medius*.

BIA	Pre Training	Post + 12 h	Post + 36 h	Post + 60 h
Z (Ω)	50.20	79.10	63.50	64.80
R (Ω)	47.10	78.90	61.20	61.70
Xc (Ω)	17.40	5.10	17.10	19.70
PA (°)	20.28	3.70	15.61	17.71

to note that *Z* and *R*, which are relatively high at the start, fall over time by some 18–21%. This is a normal occurrence as muscles immediately post exercise, typically absorb water, becoming harder and swollen (Whitehead *et al* 1998). The fall in both impedance and resistance over the 60 h post exercise denotes a return to normal muscle size and turgidity.

Horse #3: BIA recordings taken prior to surgery were used to follow the effects of a 'kissing spine' operation for a period of 5 months and to monitor muscle recovery. Measurements for both sides of the back muscles in the region (e.g. *m. Longissimus dorsi, m. Latissimus dorsi*) of the operation (T15 and T17), are given in table 4. The left-hand side of the back remains quite stable until approx. 3.5 months after surgery, when the muscle mass (*Z*) and cellular integrity (*Xc*) are affected by the period of inactivity and begin to atrophy. This continues to progress up to approx. 4 months after surgery where, after a mild form of retraining, the *Z* and *Xc* values begin to improve. The changes associated with the right-hand side of the back are a little different from those of the left-hand side. First of all, the surgeon performed the 'kissing spine' operation from the right-hand side. As a consequence this region has lost a considerable amount of muscle mass (*Z*) at 2.5 months post surgery and is showing signs of swelling (*R*). Since PA is calculated as the arc-tangent of *Xc* divided by the resistance, a large fall in *R* with a slight reduction in *Xc* will give a false increase in the phase angle. This is what we note for the right-hand side at 2.5 months post operation. This is a point to watch out for, as an increase in PA might wrongly be seen as an improvement in muscle health, if the other parameters are not checked. In addition, the right-hand side is more affected in terms of the BIA values than the left-hand side. The changes in the *Z* and *R* values, not to mention the *Xc*, of the right-hand side are more extensive than those found on the left-hand side, and they serve to highlight the skill of the surgeon in terms of localizing the damage to surrounding tissue under such an operation. However, as with the left-hand side, one can clearly see that by 5 months post operation, the right-hand side of the back of this horse is improving in terms of muscle mass and cellular integrity and that signs of tissue swelling diminishes after 3.5 months post surgery. At 5 months post surgery the left-hand side *Xc* and PA values are approx. 75 and 90% of the pre-operation values, whilst the right-hand side values are approx. 75 and 85% of the pre-operation values—these differences being minimal and in agreement with a less routinely trained horse.

Horse #4: Measurements were taken over the *m. Gastrocnemius*, see figure 1. The *Z* and *R* for the left hind leg *m.Gastrocnemius* compared with those for the right hind leg muscle are considerably elevated (see table 5). Looking at the typical BIA values, *Z*, *R*, *Xc* and PA would lead one to assume that the *m. Gastrocnemius* of the left hind leg is suffering from perfusion issues resulting in the high *R* value, a lack of nutrient provision resulting in a lower *Xc* value and the combined high *R* and low *Xc* giving rise to the low phase angle. However, a closer look at the BIA values and in particular some of the values associated with the β-dispersion range (10 kHz–10 MHz), presents a different picture. The *Ri* value is very high and the *Mc* relatively low for the left hind leg *m. Gastrocnemius* compared with the right hind leg muscle.

Table 4. Horse #3—mfBioimpedance values for the right- and left-hand back in the region of the 'kissing spine' taken prior to the operation and over a period of 5 months post recovery.

BIA	Pre—Op	Post—Op (+2.5 months)	Post—Op (+3.5 months)	Post—Op (+4.5 months)	Post—Op (+5 months)
Left-hand side					
Z (*Ω*)	48.60	45.30	40.00	35.80	40.10
R (*Ω*)	46.70	43.10	38.70	34.50	38.80
Xc (*Ω*)	13.50	13.90	10.40	9.50	10.10
PA (°)	16.20	17.87	15.04	15.40	14.59
Right-hand side					
Z (*Ω*)	46.20	30.80	29.20	34.10	39.90
R (*Ω*)	44.10	28.40	28.00	32.70	38.70
Xc (*Ω*)	13.80	12.10	8.10	9.80	10.00
PA (°)	17.38	23.08	16.13	16.68	14.49

Table 5. Horse #4—mfBioimpedance values for the left and right hind leg muscle *m. Gastrocnemius*. Note the left hind leg was the one that sustained a training related injury.

BIA	Left Hind Leg	Right Hind Leg
Z (*Ω*)	101.10	46.00
R (*Ω*)	100.40	39.41
Xc (*Ω*)	11.90	23.80
PA (°)	6.70	31.10
fc (kHz)	16.20	31.10
Re (*Ω*)	138.00	89.10
Mc (pF)	26.00	54.40
Ri (*Ω*)	242.30	4.90

The *Ri* is known to be positively correlated to VO_{2max} at rest (Stahn *et al* 2008), whilst a low *Mc* is indicative of cell membrane transport (Ivorra 2003). There is clearly evidence then that perfusion is not the underlying issue in this case, as the muscle fibres are in an anabolic state and using oxygen to repair damaged tissue. In support of this the *Re* is very low, indicating that extracellular fluid around the muscle fibres is relatively scarce. It is known that cells that are metabolically active and in an anabolic state affect the local osmotic gradients in such a way that fluid is drawn into these active cells, reducing the extracellular fluid compartment as a consequence (Sigmund *et al* 2014).

Horse #5: Measurements were made for both sides of the neck (*m. Splenius*), see figure 1 for the setup and the values are given in table 6. Once again, a quick look at the typical BIA values, *Z*, *R*, *Xc* and PA would lead one to assume that the *m. Splenius* of this horse was relatively healthy (*Xc*), if under-trained (PA). Despite its left-to-right symmetry, the horse shows signs of post-exercise muscle swelling—elevated *Z* and *R* values compared to control values. However, yet again a closer look at the BIA parameters, in particular some of the values associated with the β-dispersion range (10 kHz–10 MHz), presents a different picture. The fc values also reveal a good degree of symmetry and no obvious signs of over-contraction or -relaxation, but the *Re* values are very high, as are the *Ri* values and the *Mc* values equally low. These values, combined with the elevated *Z* and high *R* values suggest an anabolic response (*Ri* representing VO_{2max}) in the measured muscle fibres, such that an osmotic gradient has resulted in muscle cell swelling (Z) and a depletion of the extracellular fluid compartment

Table 6. Horse #5—mfBioimpedance values for the left and right neck muscle *m. Splenius*.

BIA	Left *m. Splenius*	Right *m. Splenius*
Z (Ω)	94.70	98.30
R (Ω)	93.30	97.10
Xc (Ω)	16.20	15.40
PA (°)	9.85	9.01
fc (kHz)	46.30	46.80
Re (Ω)	120.30	123.90
Mc (pF)	12.35	11.53
Ri (Ω)	157.90	171.20

(*Re*). It is well-known that for example high levels of insulin induce cell swelling due to their affect on membrane cotransporters and glucose transporters.In this way they activate anabolic processes in muscle cells (Zierler *et al* 1985, Zhao *et al* 2004). It is equally well-known that with modern carbohydrate rich diets, horses can often have an elevated plasma insulin level. However, a blood sample showed that this was not the case for this particular horse. Alternatively, a high metabolic rate induced by for example thyroid hormone imbalance would also be expected to give such results. In fact, a blood sample revealed that this subject had subclinical signs of hypothyroidism with a plasma iodine level of $29\,\mu g\,l^{-1}$, where the normal range is $50–120\,\mu g\,l^{-1}$. It has been shown in other species, that subclinical hypothyroidism can give rise to a hypersensitivity of muscle through an up-regulation of nuclear thyroid hormone receptors (Harrison *et al* 1996). Such a response can explain the observed highly elevated *Ri* and lowered *Mc*. Following three months with a feed supplement of seaweed, rich in iodine, this subject was healthy and active again.

4. Discussion and conclusions

This study has demonstrated the many applications mfBIA can be used for in the assessment of horses, both in the clinic and in the sports situation. It does not demand specialist training to apply the method and it is feasable to use the technique even in fairly primitive environments.

4.1. Interpretation of the multiple mfBIA parameters in the assessment of horses

4.1.1. Z and R. To the best of our knowledge, mfBIA has not been reported for muscles of a cohort of healthy equine subjects. This study presents a reference data set for three functional line muscle groups for a group of similarly aged, weight matched and trained horses. *Z* and *R*, when similar, may be a good measure of muscle mass, but in combination with *Xc* they can provide a PA value that becomes independent of body mass and height. In this healthy horse cohort, there are clear PA differences between the three functional line muscles—the least well developed/trained being the neck muscle (*m.Splenius*), after which comes the back muscle (*m.Longissimus dorsi*) and the most highly trained being the hip muscle (*m.Gluteus medius*) which not only plays a role in hind limb extension, but also facilitates jumping.

4.1.2. R, Xc and PA. When looking at R, *Xc* and PA values together, a powerful tool is at hand. In the case of Horse #3 (see table 4 +2.5 months), a state of oedema post surgery results in a low *R* value despite very little effect on the *Xc* value, with the result that the PA is erroneously high. Conversely, one may equally assume that a low *Xc* value for a muscle, where the resistance is close to the typical mean, will result in a false decrease in the PA. These findings in the horse are confirmed in human peritoneal dialysis patients, where changes in *R* and *Xc* with fluid removal during treatment result in similar effects on the mfBIA PA parameter (Davenport 2013).

4.1.3. Xc and Re When combining *Xc* and extracellular resistance (*Re*) values, a descriptive picture of the actual situation is at hand. Although an elevated *Re* may be indicative of dehydration, it could be due to an impaired perfusion, as seen in cardiovascular disease (Tonkovic *et al.* 2000). Note that for Horse #1 (see table 2 Pre) the *Xc* values are very low. In this particular case, the *Re* was 114 Ω compared with a normal range of 73.8 ± 5.1 (Mean ± SD; $n = 39$) and the horse was losing muscle mass, was constantly tired and had obvious problems breathing whilst standing at rest—all as a result of cardiovascular failure resulting in an inadequate perfusion of the musculature.

4.1.4. Ri, Re and Mc. In the healthy horse cohort, there is a clear difference in terms of *Re*, *Ri* and *Mc* for the three functional line muscles. The lowest *Mc* is found in the neck muscle (*m.Splenius*), and this appears in combination with an elevated *Re* and *Ri* compared with the two other muscles measured on these horses. This is perhaps not so surprising when one considers the way in which the neck is often reined in during training, thereby improving control. Combined, these values are indicative of a state of over-training in the neck muscles.

Membrane capacitance (*Mc*) shows the status of the muscle fibre membrane potential. This illustrates a combination of fibre health and transport status of the membrane. *Ri* is another important parameter concerning metabolic state, since it has been shown to be correlated with VO_2-max at rest and can therefore be interpretated as an index of anabolic status of muscles (Stahn *et al* 2008). When these parameters are considered in conjunction with the extracellular resistance, a more facetted picture of the muscle condition is at hand.

In Horse #4 (see table 5), the left hind leg sustained a serious training-related injury and the *Ri* is a consequence very high, as the muscle fibres are anabolically active in a repair state. The *Re* is also very high as the extracellular fluid has partially entered the muscle fibres—a typical post training effect (Sigmund *et al* 2014). It is also noteworthy that the *Mc* is rather low in the left hind limb of Horse #4, once again indicating a high level of cellular transport *cf* the right hind leg. In the right hind leg a low resting *Ri* value can be seen together with a normal *Mc* and *Re* value, all of which indicate a healthy and resting muscle.

4.1.5. fc. Looking at the Cole–Cole plot (showing resistance against reactance), fc is the frequency at which the maximal *Xc* is recorded (Ivorra 2003). The fc is indicative of the amount of energy needed to send 800 μA of current through the tissue. With a higher level of contraction, the greater density of the muscle tissue will need a higher energy, i.e. a frequency, to pass through the tissue. If the fc of a given muscle decreases over a period of time, it indicates that the muscle has become less tense/contracted i.e. become more relaxed. In the case of Horse #4, there is a clear difference in the fc value—lower in the left injured leg *cf* the healthy right leg, indicating a lower level of contraction in the injured leg.

Myofascial release is a soft tissue therapy for the treatment of skeletal muscle immobility and pain (Barnes 1990). The therapy relaxes contracted muscles, improves blood and lymphatic circulation and stimulates the stretch reflex in muscles. In the case of Horse #1 the myofascial treatment may directly have affected the Ruffini receptors (Macefield 2005), which respond slowly in an adapting manner to applied strain on the tissue and thereby induce the observed relaxation of the muscle.

4.2. mfBIA in assessment of metabolic state

It is known that in some species, mild hypothyroidism results in an up-regulation of thyroid hormone nuclear receptors in some organs and tissues, among them muscle, in an attempt to redress the hormone imbalance (Harrison *et al* 1996). Such an adaptive change, on brief occasions when the plasma total T_3 and T_4 as well as free T_3 levels are barely sub-clinical, will induce a hyper-metabolic state. In the case of Horse #5, where an iodine deficiency and mild sub-clinical hypothroidism was diagnosed, a similar hyper-sensitization of skeletal muscle can be expected. It is therefore interesting that the mfBIA parameters of *Ri* were elevated in the muscles measured, indicating an anabolic state (Stahn *et al* 2008). It is also noteworthy that the *Mc* is very low, indicating a high level of cellular transport. The high *Re* also supports a raised metabolic state i.e. raised body temperature and increased sweating, both of which were observed at the time of measurement. It was a combination of the *Ri*, *Mc* and *Re* values that suggested a thyroid hormone imbalance as a possible explanation for the observed fatigue and muscle wasting in this subject.

5. Conclusion

Our study of healthy horses, as well as of veterinary cases, has shown the wide scope of the application of mfBIA in assessing muscle condition and health, both when looking at progress during training schemes and in the veterinary clinic. mfBIA is non-invasive and useable in most settings and applied in full with all possible parameters measured, it is a powerful tool both in the assessment of training progress or monitoring of recovery from treatment, as well as being a diagnostic tool.

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