Universal spectral profile and dynamic evolution of muscle activation: a hallmark of muscle type and physiological state

Sergi Garcia-Retortillo[1,2,3], Rossella Rizzo[2,4], Jilin W.J.L. Wang[2], Carol Sitges[5] & Plamen Ch. Ivanov[2,6,7]

[1]University School of Health and Sport (EUSES), University of Girona, 17190, Salt, Spain.

[2]Keck Laboratory for Network Physiology, Department of Physics, Boston University, Boston, MA 02215, USA.

[3] Complex Systems in Sport, INEFC Universitat de Barcelona (UB), 08038, Barcelona, Spain.

[4]Evolutionary Systems Group Laboratory, Department of Physics, University of Calabria, Arcavacata di Rende, CS, Italy

[5]University of Balearic Islands, Department of Psychology, Research Institute of Health Sciences (IUNICS) and Health Research Institute of the Balearic Islands (IdISBa), 07122, Palma, Spain

[6]Harvard Medical School and Division of Sleep Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA

[7]Institute of Solid State Physics, Bulgarian Academy of Sciences, Sofia 1784, Bulgaria

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Muscle spectral profiles and evolution across time scales.

Corresponding Author:

Plamen Ch. Ivanov (plamen@buphy.bu.edu).

590 Commonwealth Avenue, SCI-210, Boston, MA 02215, USA.

Authors' contributions

PChI conceptualized and initiated the research. SGR and PChI designed the research protocol and methodology. SGR performed experiments and collected data from human subjects. CS provided data recording equipment and helped with data collection and results interpretation. RR, JWJLW, SGR, and PChI performed data analysis. SGR and PChI wrote the manuscript. PChI, SGR, RR, JWJLW and CS critically discussed and contributed to the paper. PChI supervised the work.

Abstract

Purpose

The skeletal muscle is an integrated multi-component system with complex dynamics of continuous myoelectrical activation of various muscle types across timescales to facilitate muscle coordination among units and adaptation to physiological states. To understand the multi-scale dynamics of neuromuscular activity, we investigate spectral characteristics of different muscle types across timescales and their evolution with physiological states. We hypothesize that each muscle type is characterized by a specific spectral profile, reflecting muscle composition and function, that remains invariant over timescales and is universal across subjects. Further, we hypothesize that the myoelectrical activation and corresponding spectral profile during certain movements exhibit an evolution path in time that is unique for each muscle type, and reflects responses in muscle dynamics to exercise, fatigue, and aging.

Methods

To probe the multi-scale mechanism of neuromuscular regulation, we develop a novel protocol of repeated squat exercise segments, each performed until exhaustion, and we analyze differentiated spectral power responses over a range of frequency bands for leg and back muscle activation in young and old subjects.

Results

We find that leg and back muscle activation is characterized by muscle-specific spectral profiles, with differentiated frequency bands contribution, and a muscle-specific evolution path in response to fatigue and aging that is universal across subjects in each age group.

Conclusion

The uncovered universality among subjects in the spectral profile of each muscle at a given physiological state, as well as the robustness in the evolution of these profiles over a range of timescales and states, reveals a previously unrecognized multi-scale mechanism underlying the differentiated response of distinct muscle types to exercise-induced fatigue and aging.

Keywords: muscle fibers, spectral power, time scales, fatigue, aging.

New & Noteworthy

To understand coordinated function of distinct fibers in a muscle, we investigate spectral dynamics of muscle activation during maximal exercise across a range of frequency bands and time scales of observation. We discover a spectral profile that is specific for each muscle type; robust at short, intermediate, and large time-scales; universal across subjects, and characterized by muscle-specific evolution path with accumulation of fatigue and aging, indicating a previously unrecognized multi-scale mechanism of muscle tone regulation.

1. Introduction

The skeletal muscle is a complex system composed of multiple muscle fibers, which respond individually and differently to a myriad of environmental influences (75). According to their specific myosin heavy chain expression, muscle fiber types range from slow/oxidative to fast/glycolytic (7, 8, 63) and present particular frequency profiles in response to fatigue. The literature on the use of frequency-domain parameters assessing skeletal muscle fatigue is extensive. However, there is limited research focusing on the evolution in time of the spectral power profile of frequency bands representing different muscle fibers activation, and the specific contribution of different muscle fibers frequency bands in response to exercise-induced fatigue and aging. Thus, the underlying multi-scale regulatory mechanism remains not understood.

Mean frequency and median (center) frequency are the traditionally utilized physiological measures to evaluate skeletal muscle fatigue in electromyographical EMG signals (12, 67). However, the lack of reproducibility of such frequency-domain measures, for different muscle groups, across subjects, and experimental protocols, raises questions regarding their clinical utility in assessing skeletal muscle function (10, 58, 80). Moreover, these traditional measures cannot provide complete information on how the spectral profiles of muscle activation are modulated as a consequence of fatigue. For instance, fatigue-related decrease in EMG center frequency could be provoked by an increase in low frequency power, a decrease in high frequency power, or a combination of both (3).

An alternative method to assess muscle fatigue is to measure responses in the spectral power of different EMG frequency bands (10, 16, 23, 49, 65, 72, 77). Since muscle fatigue elicits specific changes in the spectral power for different EMG frequencies (15, 73), frequency band analyses enable more detailed characterization of the response of different muscle fibers in a given muscle, as well as of different muscle types. Investigating separately the spectral intensity of low- and high-frequency EMG components, has helped to determine the different contribution levels of slow- and fast-twitch muscle fibers (31), with recent applications to muscle fatigability (24, 26), diagnosis of patellofemoral pain syndrome (23), changes in voluntary effort (61), and joint positional variability (49). Further, EMG frequency content and related spectral power characteristics have been utilized to study age-associated changes in neuromuscular control and assess sarcopenic muscle function (10).

Previous works in the field have mainly focused on separate ‘snapshots-in-time’ to quantify spectral power characteristics of frequency bands, and did not investigate how spectral profiles of muscle activation in different muscle types evolve in time in response to fatigue and age-related neuromuscular degeneration. However, fatigue- and age-induced physiological adaptations of skeletal muscle and muscle fibers continuously evolve as a result of soft-assembled states dwelling at different time scales and levels of biological system organization (36, 41, 81). Earlier studies have identified the presence of long-range power law correlations in wrist locomotion (37, 40), and gait dynamics (30) and related cardiovascular variables (44, 86) with invariant behavior at different time scales, indicating the presence of multi-scale mechanisms underlying neural regulation of locomotion (1, 38). Therefore, since muscle activation is necessary for locomotion, and given that locomotion is characterized by scale-invariant characteristics over a broad range of time scales, we hypothesize that muscle activation will also exhibit scale-invariant profiles. More specifically, we hypothesize that: 1) there is a particular evolution process in time that underlies muscle activation and related spectral power characteristics; 2) each muscle type is characterized by a spectral profile hat exhibits a muscle-specific evolution of different frequency bands in response to exercise-induced fatigue; 3) different muscle fibers within a given muscle are associate with specific time evolution paths of their spectral profiles; 4) spectral profiles of muscle activation exhibit similar characteristics across time scales; and 5) while the functional form of the spectral profile characterizing muscle activation maybe similar in young and old subjects, old subjects exhibit a different evolution path with less pronounced increase of spectral power in response to exercise and fatigue. Establishing consistency in the spectral power profiles of different muscle types and robust evolution path of these profiles over a range of time scales for all subjects in a given age group, would reveal a universal behavior related to a basic mechanism of muscle tone regulation in response to exercise-induced fatigue.

To test our hypotheses, we develop a protocol which allows us to identify and track simultaneously the evolution of the spectral power profiles of different muscle types, and to study the multi-scale mechanism underlying the differentiated response of different frequency bands to exercise-induced fatigue in young and old adults. Given that previous research on frequency banding mainly considered muscles in an isolated manner and by means of simple movements over short time segments, and because of the need to establish the relative contribution of trunk muscles together with leg muscles during more complex tasks and how it changes over prolonged periods of extended and repeated exercises (76), we use a protocol that includes repeated long squat exercise segments performed until exhaustion and interspersed by rest segments. The squat test can be considered as an administrable and reliable tool to simultaneously assess the activation of different muscles types and to measure the physical status in both young (5, 53) and old subjects (90). We collect EMG data from two different muscle types: the erector spinae back muscle composed of slow oxidative type I muscle fibers (9), and the vastus lateralis leg muscle composed of higher percentage of fast glycolytic type II muscle fibers (64, 83), both of which show high myoelectrical activity during squats but with different levels of activation and contribution to the exercise effort (46).

Accordingly, we investigate the leg (vastus lateralis, VL) and back (erector spinae, ES) muscle spectral power profiles and their time evolution during three consecutive squat tests performed until exhaustion and four interspersed rest segments, in healthy young and old adults. By quantifying the contribution of differentiated frequency bands to the spectral profile of the leg and back muscle, our study focuses on the evolution of these profiles in response to accumulated and residual fatigue over long, intermediate, and short time scales, i.e., across consecutive exercise and rest segments, within exercise segments, and for a single squat movement.

2. Methods

2.1. Participants and inclusion criteria

To determine the sample size for this study a power analysis was conducted using G∗Power 3.1 (22). Previous research assessing fatigue effects on repeated exercise performed until exhaustion (25) have reported large effect sizes. Thus, using an effect size of d = 1.2, $\alpha < 0.05$, power (1—β) = 0.80, we estimated a minimum sample size = 20. Accordingly, fourteen healthy young adults (six males and eight females; age 22.19 ± 13.56 years, height 174.69 ± 10 cm, and mean body mass 66.81 ± 13.39 kg) and seven healthy old adults (three males and four females; age 56.2 ± 2.95 years, height 169 ± 11.93 cm, and mean body mass 73.42 ± 11.09 kg), were recruited to participate in the study. With the aim of ensuring a homogenous sample, participants were strictly recruited according to the following inclusion criteria: a) aged 20-30 years (healthy young adults group) or 50-60 years (healthy old adults group), b) BMI (in kg/m^2) > 18.5 and < 30, c) normal physical activity > 5 and < 10 h/week, but without sport specialization, and d) blood pressure < 140/90 mmHg. Exclusion criteria consisted of: a) intake of prescribed drugs that could affect muscle strength, such as corticosteroids, b) current or previous injury, either during the previous period before testing or at any other moment, going against the study protocol, and c) any other condition that may have prevented the performance of an exercise protocol until exhaustion. The experiment was approved by the local ethical committee and carried out according to the Helsinki Declaration. Before taking part in the study, participants read the study description and risks and signed an informed consent (88).

2.2. Study design and test protocol

In our protocol the participants visit the laboratory for two different sessions, separated by a 2-day interval. During the first session (i.e., familiarization), participants practice the squat test until they are able to execute the movement according to the protocol (see study test protocol below). In the second session, participants perform the study test protocol.

We specifically select the squat exercise because it demands the coordinated activity between lower back and leg muscles, and has been recognized as a functional and safe movement that closely resembles complex everyday tasks (14). Furthermore, the squat is one of the most traditional resistance exercises used to enhance performance in sports and in lower limb rehabilitation processes, as it develops powerful muscles that are activated during many functional tasks, such as running or jumping (21). Since the focus of our manuscript is to identify spectral profiles of muscle activation that are specific for each muscle type, and to track the evolution of this muscle-specific spectral profiles in response to exercise-induced fatigue, we utilize a maximal (i.e., squats performed until exhaustion) instead of a submaximal squat test. The use of a submaximal squat test would not provoke sufficiently high levels of muscle fatigue, necessary to investigate changes in the spectral profiles of leg and back muscle activation and how these profiles evolve in the process of exercise from short to large time scales.

The protocol is composed of the following consecutive segments: a) 10-minute rest period in supine position (Rest 1), b) squat test performed until exhaustion (Exercise 1), c) 10-minute rest period in supine position (Rest 2), d) squat test performed until exhaustion (Exercise 2), e) 10-minute rest period in supine position (Rest 3), f) squat test performed until exhaustion (Exercise 3), and g) 10-minute rest period in supine position (Rest 4).

Rest segments. During Rest 1, 2, 3, and 4, participants lay down in a supine position on a massage table. With the aim of avoiding joint compression and facilitating relaxation, we place a pillow under the participants' knees. Furthermore, we locate another pillow under the back to avoid contact between the back electrodes and the table.

Exercise segments. During Exercise 1, 2, and 3, participants perform a squat test until exhaustion. The squat tests are performed according to the following instructions: "*Place feet a little wider than shoulder-width apart. Extend the arms out straight. Initiate movement by inhaling and unlocking the hips, slightly bringing them back. Keep sending hips backward as the knees begin to flex. Squat down until touching the rope. Return to standing position. Repeat until exhaustion.*" The rope is adjusted to a height where the participants' thighs are parallel to the ground at the bottom of their squat. Participants are instructed to keep their chest up, weight over the heels and not to allow their knees to fall into a valgus position. (5,53,78). Given that the back squat is used much more commonly compared to its front squat variation (89), and since the front squat requires higher ankle mobility (loss of ankle dorsiflexion is a common feature in young and old population; 69), the back squat has been selected for the current study. The pace of the squat is controlled by means of a metronome (MetroTimer version 3.3.2, ONYX Apps), using a 3:3 tempo (three seconds down and 3 seconds up, so one single squat lasts 6 seconds). The squat test finishes when participants are not able to squat down/up anymore or, alternatively, when they cannot maintain the prescribed squat tempo.

The repetition of three consecutive squat tests performed until exhaustion, allows us to identify the effects of acute fatigue on the leg and back spectral power profiles, and to track the evolution of the spectral power profiles with gradual accumulation of fatigue within each exercise segment. The short 10-min resting periods in our protocol lead to only a partial recovery after a maximal squat test, and allow to quantify the effects of residual fatigue reflected on spectral power profiles of leg and back muscle activation across consecutive exercise segments. While acute fatigue occurs when the energy consumption exceeds the muscle aerobic capacity and a large fraction of the required energy has to come from anaerobic metabolism (11), residual fatigue is characterized by neuro-mechanical and biochemical alterations (e.g., decrease in maximal force) provoked by previous exercise (29).

2.3. Electromyography (EMG) acquisition

Participants are asked to wear appropriate clothing for access to the EMG electrode placement sites. Before the mounting of the EMG electrodes, the participants' skin is shaved and cleaned using alcohol, and left to dry for 60s to reduce the myoelectrical impedance, according to the SENIAM guidelines (33). The following muscles are investigated simultaneously during the whole study test protocol: left and right vastus lateralis (VL-L, VL-R), and left and right erector spinae (longissimus; ES-L, ES-R). The placement of the surface electrodes (Ag / AgCl bipolar surface electrodes, Sorimex, Toruń, Poland) is also carried out according to the recommendations of SENIAM organization and the Cram Guidelines (13). More specifically, vastus lateralis electrodes are placed at 2/3 on the line from the anterior spina iliaca superior to the lateral side of the patella, and the erector spinae electrodes are located at a 2-finger width lateral from the proc. spin. of vertebra L1. After the electrodes are secured, a quality check is performed to ensure EMG signal validity. The aforementioned muscles have been selected since they present the highest myoelectrical activity during bodyweight squat (46).

2.4. EMG signal processing and data analysis

We record data using Biopac MP36 (Biopac Systems Inc, Goleta, CA, USA) and process them by means of Matlab (Mathworks, Natik, MA, USA). Raw data is recorded at a sample frequency of 500 Hz and filtered online using a 5–250 Hz band-pass filter. Furthermore, we use a notch filter with a width of 1 Hz at the frequency of 50 Hz (i.e., 49.5 – 50.5 Hz) to remove line interference.

The procedure we follow to process the data for the current research is composed of three main steps, over longer to shorter time scales. While the first step focuses on the overall spectral power changes across the whole study test protocol (i.e, larger time scale), the second and third steps aim at performing a more in-depth analysis on the changes within exercise segments (i.e., shorter time scales). All the analyses are carried out separately for both young and old groups.

The first step is to study spectral power S(f) distribution profile for both leg and back muscles and its evolution across different Rest and Exercise segments (i.e., large time scales). We extract spectral power for each muscle (i.e., VL-L, VL-R, ES-L, and ES-R) and segment (i.e., Rest 1, 2, 3, 4, and Exercise 1, 2, 3), considering a 2-second time window with an overlap of 1 second. For each time window, we compute spectral power across all frequencies. Given that no remarkable differences are observed between left and right leg and back in the current study, we only show the results for VL-R and ES-R. Next, in order to quantify the results observed in the spectral power distribution curves (Figs. 2 and 3), we compute the total spectral power $\tilde{S}(f)$ for each muscle and exercise-rest segment (Fig.4), summing up the power across all frequencies:

$$\tilde{S}(f) := \sum_{i=1}^{N} S(f_i)$$

where f_i are all frequencies considered in our spectral analysis. We obtain a value for the spectral power for each 0.5 Hz in the [5-250 Hz] range, therefore $N = 500$. Furthermore, to elucidate the contribution of different frequencies, we then subdivide the spectrum of frequencies in the following bands: [5-25 Hz], [25-50 Hz], [50-150 Hz], [150-250 Hz], and take the average spectral power corresponding to the frequency bins of 0.5 Hz in each frequency band (Fig. 5):

$$< S(f) > := \sum_{i=1}^{M} S(f_i) \Big/ M$$

where f_i are all the frequencies in each frequency band binned in bins of 0.5 Hz. Note that because the frequency bands have different width, we use the average spectral power $< S(f) >$ instead of the sum. The aforementioned frequency bands were selected according to the shape of the empirical spectral power distribution observed in Figs. 2 and 3, and relates to earlier studies of different muscle fiber types (31).

The second step of our analysis is to study the spectral power profile evolution within Exercise segments (i.e., intermediate time scales) by means of a spectrogram (Fig. 6). With the aim of further clarifying the contribution of different frequencies, we consider in this case 10 x 10 Hz frequency bands, from 5 to 200 Hz. The 200-250 Hz range was removed given the lack of activity observed in the previous figures. As in the previous step, we consider a 2-second time window with 1-second overlap. For each time window and frequency band, and calculate the sum $\tilde{S}(f)$ of all the power across all frequencies within that frequency band. Each node in Fig. 6 is assigned a color and represent the power inside the corresponding time window and frequency band. To facilitate visual comparison among exercise segments and between age groups, the same color bar ranges are used in the different subplots. The maximal power in the color bar corresponds to the highest power value obtained during the three exercise segments. In order to quantify the results shown in Fig. 6, we specifically compare the Beginning versus the End of each exercise segment. To this end, we consider 1-minute segment after the first 30 seconds (i.e., Beginning), and 1-minute segment before the very last 12 seconds (i.e., End) of each Exercise segment. The first 30 seconds (i.e., five squats) are not considered since participants needed an average of two or three repetitions to get synchronized with the metronome. The last 12 seconds (i.e., 2 squats) are also not considered given the high instability that typically characterizes the very last squats of the exercise segments due to exhaustion. We compute spectral power using a 2-second time window with an overlap of 1 second, for both Beginning and End. We consider three of the four original frequency bands (i.e., [5-25 Hz], [25-50 Hz], and [50-150 Hz]). The [150-250 Hz] band was removed from the analysis given its reduced activity in previous steps. We consider the average <S(f)> of the spectral power of all frequencies inside each frequency band (Figs. 7 to 10, right panel). To probe detailed characteristics of the spectral power profiles and the specific contribution of different muscle fibers in response to fatigue and aging, we also consider 34 frequency bands each with width of 4 Hz (from 4-44 Hz and from 56-152 Hz) for both Beginning and End of each exercise segment. The 44-56 Hz range is not included because of the notch filter at 50 Hz, which modifies the EMG signal, altering the spectral power of frequencies around 50 Hz (i.e., 49.5 – 50.5 Hz). Since the detailed frequency bands have the same width of 4 Hz, we next calculate the sum of the spectral power $\tilde{S}$(f) for all frequency bins of 0.5 Hz inside each frequency band (Figs. 7-10, left panels). Note that, given the lack of remarkable differences between Exercise 2 and 3, Exercise 2 is not shown from Fig. 6 onwards.

The last step of our analysis is to identify the spectral power profiles during single squat movements (i.e., short time scales), and analyze the evolution of these profiles from Beginning to End of the Exercise segments. Thus, we take five squats from the Beginning (i.e., squat 6 to 10) and five squats from the End (i.e., the last five squats, without considering the very last two squats) of Exercise 1. We next divide each squat into two parts: Down (3 seconds; lengthening contraction) and Up (3 seconds; shortening contraction). Then, we compute the spectral power for both Down and Up (Beginning and End), considering a 1-second time window with an overlap of 0.5 seconds. Similar to the previous step, we consider both the three original frequency bands ([5-25 Hz], [25-50 Hz], and [50-150 Hz]) and the 34 frequency bands (from 4 to 44 Hz and from 56 to 152 Hz, each 4 Hz). We next compute the sum $\tilde{S}(f)$ (34 frequency bands; Figs. 11 and 12) or the average $<S(f)>$ (4 original frequency bands; Figs. 13a,b and 14a,b) of the power across all frequencies inside each frequency band. Finally, in order to study the evolution of lengthening and shortening contractions from Beginning to End, we obtain the ratios $<S(f)>_{End}/<S(f)>_{Begin}$ (Figs. 11c and 12c). The ratios are obtained dividing Down phase Beginning by Down phase End, and Up phase Beginning by Up phase End values of the averaged spectral power for each frequency band.

The selection of short 1-min time period (Figs. 7-10; intermediate time scales) or 5 squats (Figs. 11-14; short time scales) at the Beginning and End of each exercise segment, allows for more accurate quantification of the spectral profiles of the leg and back muscle activation for different physiological states within exercise segments: absence of fatigue at Beginning of Exercise 1, residual fatigue at Beginning of Exercise 2 and 3, and maximal level of fatigue accumulation at End of each exercise segment. Selection of longer time periods within exercise segments would lead to reduced accuracy in the analysis to quantify association of distinct physiological states with the spectral power profiles of muscle activation for different muscle types and age groups - absence of fatigue can be accurately tested only at the Beginning of Exercise 1, residual fatigue only at the Beginning of Exercise 2 and 3, and maximum level of accumulation of fatigue only at the End of Exercise 3. Alternatively, a choice of shorter time periods to quantify these physiological states would not provide sufficient data to perform reliable analyses.

2.5. Statistical tests

Statistical analyses are performed using SPSS (v.23, SPSS Inc., USA). All data are tested for normality by using a Shapiro-Wilk test. To analyze the spectral power frequency bands evolution across Exercise 1, 2, and 3 (Figs. 4 and 5), we perform a repeated measures ANOVA with Bonferroni post-hoc correction separately for each muscle. To assess the changes within exercise segments (i.e., Beginning vs End; Figs.7-10) and during a single squat movement (Down vs Up squat phase; Figs. 11-14) we use a Student-t test. The between-group comparison (young vs old) is computed by means of an independent Student-t test. Alternatively, in case of non-Gaussian distribution, we use a Friedman ANOVA (across exercise segments), a Wilcoxon matched pairs test (within exercise segments and during a single squat movement), or a Mann-Whitney U matched pair test (between age groups). We use an alpha level of 0.05 for all statistical tests.

3. Results

3.1. Performance

The number of squats significantly decreases across the three exercise segments, in both the young (123.50 ± 41.73, 53.64 ± 19.95, and 47.54 ± 23.39 in Exercise 1, 2, and 3, respectively; Friedman ANOVA test; $\chi^2 = 22.29$; df = 2; $p < 0.001$) and the old group (65.23 ± 36.82, 39.51 ± 24.02, and 32.00 ± 20.85, in Exercise 1, 2, and 3, respectively; Friedman ANOVA test; $\chi^2 = 14.00$; df = 2; p = 0.001). Specifically, the number of squats is significantly reduced from Exercise 1 to Exercise 2 (Wilcoxon matched pairs test; $Z = 3.29$; $p = 0.01$ for the young and $Z = 2.36$ $p = 0.01$ for the old group. In the between-group comparison, the young subjects perform significantly more squats than the old group only in the Exercise 1 (Mann Whitney U test; $U = 13.00$; $p = 0.006$).

3.2 Spectral power distribution of leg- and back-muscle activation for consecutive rest and exercise segments

The leg and back muscles show different EMG amplitude profiles at both large and intermediate, and short time scales (Fig. 1). Within and across exercise segments, there is a progressive increment in the EMG amplitude of leg, reflecting the effect of fatigue, in contrast to the back muscle where the EMG amplitude does not remarkably change. Note also the higher initial EMG amplitude at the Beginning of Exercise 2 and 3 compared to Exercise 1 for the leg muscle indicating residual fatigue – an effect that is not present for the back muscle (Fig. 1a). The leg and back muscles also show markedly distinct EMG amplitude profiles at short time scales of a few seconds, associated with individual squats. While the leg muscle presents a bimodal profile with two phases corresponding to the down (smaller amplitudes) and up (larger amplitude) squat movements, the back muscle shows a unimodal EMG profile (Fig. 1b). The observed differences at both short and large time scales between the leg and back muscles in the EMG amplitude profiles and its evolution with fatigue, indicate different muscle fiber structure and role during the squat. These empirical observations motivate our hypothesis that distinct muscles have specific spectral power profiles, with different contribution of muscle fibers to the spectral power of high- and low- frequency components, and muscle-specific spectral power evolution profiles across short and large time scales in response to exercise-induced fatigue and aging.

To test our hypothesis, we first obtain the spectral power distribution for the leg and back muscles, during rest and exercise, and for both young and old subjects. Our analysis shows that the leg and back muscles have different spectral power profiles, according to their specific histochemical properties and distinct role during the squat movement (Fig. 2). Specifically, while both leg and back spectral profiles exhibits a major contribution of low frequencies, the leg spectral profile is also characterized by a more remarkable contribution of higher frequencies compared to the back muscle. These muscle spectral profiles are also different during rest and exercise, indicating specific muscle fiber contribution during different physiological states (rest vs. exercise). Note also that both leg and back spectral profiles are preserved in the old group, however, with a reduced total power with age, reflecting the typical decline in muscle mass (sarcopenia) and strength in old adults (91).

3.3. Leg- and back-muscle spectral power profiles and their evolution at large time scales across consecutive rest and exercise segments

We next ask the question whether the spectral power profiles of the leg and back muscles evolve and change at large time scales across consecutive rest and exercise segments. We remarkably find that the shape of the spectral profiles does not change across rest (Fig. 2a,b) and exercise segments (Fig. 2c,d), however, there is a marked vertical shift to higher spectral power across exercise and rest segments. According to the performance results (Methods 3.1.), this $S(f)$ vertical shift present in both leg and back muscle, is more pronounced between Exercise 1 and 2 than from Exercise 2 to 3, and reflects the response to residual fatigue.

Moreover, we investigate whether the observed spectral profiles in the leg and back muscle and their evolution with aging is different (Fig. 3). We observe that young and old subjects show similar evolution in the leg and back muscle spectral profiles. These observations indicate that the spectral power profiles for both leg and back muscle are robust as they are consistently reproduced at a given physiological state (rest - exercise) and muscle (leg - back) and are preserved across rest and exercise segments.

Rest segments. To quantify the results observed in Fig.1 and Fig. 2, we compute the total spectral power for each muscle during the four rest segments (Fig. 4a,b). A significant effect of accumulated fatigue is only observed on the right back muscle total power, in both young (ANOVA repeated measures; F= 3.98; df = 3; *p* = 0.02; Exc. 1 vs Exc. 2 p = 0.02) and old (F = 5.92; df = 3; *p* = 0.005; Exc. 1 vs Exc. 2 p = 0.03) groups. Further, to elucidate the contribution of low-and high-frequency components to the total spectral power, we quantify the power in the following frequency bands: [5-25 Hz], [25-50 Hz], [50-150 Hz], and [150-250 Hz], and their evolution across rest segments (Fig. 5a). A significant effect of accumulated fatigue is mainly shown in the low frequency [5-25 Hz] band (F = 3.47; df = 3; p < 0.02), indicating a clear dominance of the low frequencies during the four rest periods. Note that the other frequency bands were mostly absent in both the right leg (vastus lateralis, VL-R) and right back (erector spinae, ES-R) muscles. According to the size principle of motor recruitment (32,56), type I muscle fibers are recruited at rest and during light exercise, provoking a clear dominance of low frequencies during rest segments. As shown in Fig. 5a, the [5-25 Hz] band is about 10^2 times higher in the ES-R compared to the VL-R. A feasible explanation could be related to the different fiber composition in VL and ES muscles; while ES is a postural muscle mainly composed of type I fibers (9), VL has a higher percentage of type II fibers (64,83). This means that the ES might present higher muscle tone at rest, provoking an increased spectral power, specifically at low frequencies. When comparing between age groups, the [5-25 Hz] band is significantly higher in the young group during the four Rest segments, specifically in the ES-R (Fig. 5a; t = 1.72; p = 0.04). No significant differences are shown between age groups regarding the VL-R.

Exercise segments. With the aim of quantifying the leg and back spectral power evolution across exercise segments observed in Fig. 2, we next compute the total spectral power for each muscle during the three consecutive exercise segments (Fig. 4c,d). With transition from rest to exercise, both the right leg (vastus lateralis, VL-R) and the right back (erector spinae, ES-R) total spectral power increase 10^2 to 10^3 times, in young and old subjects. The VL-R activation in both age groups shows significant effect of accumulated fatigue with 36% increase in spectral power for young (ANOVA repeated measures; F = 9.78; df = 2; p = 0.001; Exc. 1 vs Exc. 2 p = 0.01) and 40% increase for old subjects from Exercise 1 to Exercise 2 (F = 9.06; df = 2; p = 0.004; Exc. 1 vs Exc. 2 p = 0.04). In contrast, the ES-R exhibits significant evolution in spectral power across exercise segments with 23% increase from Exercise 1 to Exercise 2, only for the young subjects (ANOVA repeated measures; F = 4.72; df = 2; p = 0.03; Exc. 1 vs Exc. 2 p = 0.04). No significant differences are observed for the total spectral power of the VL-R activation between young and old subjects during exercise. However, the ES-R total power is significantly higher in young than old subjects (Student t-test p < 0.01). The reduced ES-R power evolution in the old group might be explained by the typical decline in lumbar extensors strength, which has been observed from the third to sixth decade of life (20,77). Given the usual higher myoelectrical activity (i.e., higher contribution) of VL compared to ES muscle during squats (46), total power is 100% higher in the VL-R compared to the ES-R, in both young and old groups.

As for the previous subsection (Rest segments), we also identify the specific contribution of different frequency bands to the VL-R and ES-R total power (shown in Fig. 4c,d) for consecutive exercise segments (Fig. 5b). Spectral power increases (~30%) proportionally for all frequency bands, in both young and old groups, reflecting common response across the entire frequency range. Specifically, a significant effect of accumulated fatigue on VL-R is observed in all frequency bands in the young group (ANOVA repeated measures; df = 2; $p < 0.03$), and only in the [5-25 Hz] and [25-50 Hz] bands in the old group (ANOVA repeated measures; F= 7.11; df = 2; p = 0.09 and F = 5.35; df = 2; p = 0.02, respectively). In contrast to the VL-R, a significant effect of accumulated fatigue on the ES-R is observed in [5-25 Hz] and [25-50 Hz] bands (ANOVA repeated measures; F = 4.57; df = 2; p = 0.02 and F = 4.52; df = 2; p = 0.02, respectively) for the young subjects, and only in [5-25 Hz] band for the old subjects (ANOVA repeated measures; F = 4.72; df = 2; p = 0.04). According to the size principle of motor recruitment (32,56), during exercise segments both the [5-25 Hz] and [25-50 Hz] bands dominate and the [50-150 Hz] band is remarkably present, indicating the recruitment of faster motor neurons with higher excitation thresholds (84), compared to the rest segments. Furthermore, given the distinct muscle fiber composition of VL and ES muscles, a significant reduction in spectral contribution in the intermediate [25-50Hz] frequency band compared to the low frequency [5-25Hz] band is observed in the ES-R for the thee exercise segments (Student t-test; t = 5.04; $p < 0.001$; t = 3.95; p = 0.002, and t = 4.02; p = 001), an effect which is not observed for the VL-R. As shown in previous studies, the high- and low-frequency contents within the EMG seem to be associated with the recruitment of fast and slow motor units, respectively (4, 34, 68, 85). More specifically, the shape and conduction velocity of the motor unit action potentials, are determined by the intrinsic attributes of muscles fibers (6,84) that make up each motor unit, forming the basis for the spectral properties in the EMG. Thus, since ES is mainly composed of type I fibers (9), which seem to be recruited below 40-50 Hz (17,87), there is a less pronounced response in the [25-50 Hz] band and no evolution is observed in the [50-150 Hz] and [150-250 Hz] bands.

The consistency of the spectral power profiles among subjects from a given group (young - old), at a given physiological state (rest - exercise) and muscle (leg – back), and the robustness of these profiles at large time scales for repeated rest and exercise segments, indicate a universal behavior related to a basic mechanism of muscle tone regulation.

3.4. Leg- and back-muscle spectral profiles and their evolution at intermediate time scales within a single exercise segment

Further, we ask the question whether the robustness of the leg and back spectral profiles observed at large time scales in both young and old groups (Fig. 4 and Fig. 5), is preserved at intermediate time scales during Exercise 1 with accumulation of fatigue (Figs. 7a, 8a, 9a, 10a). Moreover, we test how residual fatigue from Exercise 1 and Exercise 2 affects the shape and evolution of such spectral profiles during Exercise 3 (Fig. 7b, 8b, 9b, 10b).

Leg muscle spectral power evolution with accumulation of fatigue. With progression of the exercise in the young group, there is a clear evolution in the leg muscle (vastus lateralis, VL-R) spectral profile from fewer active frequency bands (mainly low frequencies, below 50 Hz) with relatively lower spectral power at the Beginning of Exercise 1, to a broader range of frequency bands (both low and high frequencies, above 50 Hz) at higher level when approaching exhaustion at the End of the exercise (Fig. 6a). As stated by previous authors (56), motor units are recruited from smallest to largest. This means that performing submaximal squat contractions results mainly in the recruitment of lower threshold motor units that innervate type I fibers, but increasing fatigue leads to the recruitment of higher threshold motor units that innervate type II muscle fibers. Accordingly, VL-R spectral profiles in the young group are characterized by two separate Regimes with different response to accumulating fatigue in the course of exercise (Fig. 7a, left panel). Regime 1, containing low [5-25 Hz] and intermediate [25-50 Hz] frequency bands, increases dramatically up to 200% from the Beginning to End of exercise (Wilcoxon matched pairs test; $Z = 3.30$; $p = 0.001$ and $Z = 2.93$; $p = 0.003$). Regime 2, which includes high frequency bands [50-150 Hz], shows a less pronounced increase in spectral power at the End of exercise (approximately 100%; Wilcoxon matched pairs test; $Z = 3.29$, $p = 0.001$). Thus, a clear dominance of lower frequencies as the muscle becomes fatigued can be observed. According to previous research, the main factor leading to the low-frequency dominance profile are the changes in the shapes of the motor unit action potentials, which are caused primarily by a reduction in the conduction velocities of the active fibers (3, 62). In turn, the conduction velocities are largely influenced by intracellular pH (56) and acidosis developed during high-intensity exercise. The maximal squat test is a highly demanding exercise, in which elevated levels of acidosis are accumulated when approaching exhaustion (42).

Regarding the VL-R spectral power evolution within Exercise 1 in the old group (Figure 9a), a very similar S(f) profile and evolution from Beginning to End of the exercise (in both individual subject and group average) can be observed, compared to the young group (Figure 7a). The spectral power profile also exhibits two distinct regimes of frequency bands (Figure 9a, left panel), with (i) different starting levels of power at the Beginning of exercise and (ii) distinct response to accumulation of fatigue with progression of exercise. Concretely, there is a more pronounced increase in the low [5-25 Hz] and intermediate [25-50 Hz] bands in Regime 1 (Wilcoxon matched pairs test; $Z = 2.02$; $p = 0.04$ and $Z = 2.12$; $p = 0.03$) compared to high frequencies [50-150 Hz] in Regime 2 ($Z = 1.63$; $p = 0.04$). The observed leg muscle spectral power profile and its evolution with fatigue in the old group (Fig. 9a) is consistent with the leg muscle spectral response of young subjects in Fig. 7a, indicating universality in myoelectrical activation during exercise. However, in contrast to the young group, the spectral power of low and intermediate frequency bands (Regime 1) in old subjects starts at significantly higher levels at the Beginning of Exercise 1 (with approximately 30%; Mann Whitney U test; $U = 51.00$; $p = 0.04$), indicating a reduced frequency range response during exercise. Furthermore, while the increase in Regime 1 at the End of exercise is less pronounced (50% for [5-25 Hz] and 50% for [25-50 Hz] in old, vs. 220% and 110% respectively in young subjects), the increase in high frequencies [50-150 Hz] in Regime 2 is completely absent in the old group. The overall reduced spectral power and the lack of activity at higher frequencies in the old compared to the young group, might be explained by the reduction in the discharge rate of motor neurons in old adults (48), and by sarcopenia, the normal decline of skeletal muscle and strength during aging (2, 91). Sarcopenia involves primarily type II muscle fibers, which motor units seem to fire above 50 Hz and up to 100-140 Hz (28,87).

Leg muscle spectral power response to residual fatigue. A very similar spectral profile is observed for the leg muscle (vastus lateralis, VL-R) in the young group within Exercise 3 (Fig. 7b), compared to Exercise 1 (Fig. 7a). However, the existent significantly elevated spectral power in Regimes 1 and 2 at the Beginning of Exercise 3 compared to Exercise 1 (Wilcoxon matched pairs test; $Z = 2.90$; $p = 0.04$), indicates the presence of residual fatigue from Exercise 1 and 2. This residual fatigue effect leads to a reduced response to accumulated fatigue during Exercise 3 compared to Exercise 1 in young subjects: approximately 50% increase for the [5-25 Hz] and [25-50 Hz] bands for Exercise 3 (Wilcoxon matched pairs test; $Z = 3.15$; $p = 0.002$ and $Z = 3.17$; $p = 0.001$) compared to 240% and 170% respectively for Exercise 1 (Fig. 7). Note also that the level of power response in the Regime 1 frequency bands at the End of Exercise 3, corresponding to the maximum leg muscle capacity is similar than in Exercise 1. In contrast to Regime 1, no significant increments from Beginning to End during Exercise 3 are detected in the high frequencies [50-150 Hz] band in Regime 2 in young subjects. The lack of increment at high frequencies in Exercise 3 might be related to the greater fatigability characterizing type II fibers (11,63).

The aforementioned behavior in Regime 1 and Regime 2 for young subjects, in response to accumulated fatigue during Exercise 3 and in response to residual fatigue is consistently observed in the old group (Fig. 9b). As in the young group, the power at the Beginning of Exercise 3 (Fig. 9b) is increased about 50% compared to Exercise 1 (Fig. 9a). However, the increase from Beginning to End of Exercise 3 is less pronounced in the old group (30% increase for [5-25 Hz] and 20% for [25-40 Hz] bands; Wilcoxon matched pairs test; $Z = 2.37$; $p = 0.02$ and $Z = 2.21$; $p = 0.03$) compared to young subjects, indicating a reduced response of VL-R activation during Exercise 3.

Note that the observed evolution in the VL-R spectral profile at intermediate time scales during exercise (Figs. 6, 7, and 9) in response to accumulated fatigue, is consistent with the results shown for the change in the VL-R spectral power at large time scales across consecutive exercise segments (Figs. 4c,d and 5b), where the power increases from Exercise 1 to Exercise 2, but does not change from Exercise 2 Exercise 3 due to effects of residual fatigue.

Back muscle spectral power evolution with accumulation of fatigue. As for the leg muscle (VL-R), the back muscle (erector spinae, ES-R) spectral profile is also characterized by two distinct frequency regimes with different response to accumulating fatigue in the course of exercise (Fig. 8a). However, in contrast to the leg muscle, ES-R spectral power in high frequency bands (above 55 Hz) does not increase during exercise, and response to fatigue with widening range of active frequency bands and increased power is observed only for low and intermediate frequencies (Fig. 6a). Specifically, a significant effect of accumulated fatigue from Beginning to End in the young group is only observed on Regime 1 – 140% increase in S(f) in the low [5-25 Hz] and 70% in the intermediate [25-50 Hz] band (Wilcoxon matched pairs test; Z = 3.05; p = 0.02 and Z = 2.42; p = 0.01) (Figure 8a). The lack of changes on the high frequencies [50-150 Hz] in Regime 2 for the back spectral profile, show a remarkable dissociation in response to fatigue of back muscle fibers represented by different frequency bands, given the specific back muscle fiber composition (9).

The spectral power profile of the back muscle and its evolution with fatigue in the old group (Fig. 10a) are consistent with the young subjects in Fig. 8a, indicating universality in myoelectrical activation during exercise. However, there is an overall remarkable reduction (10 times lower) compared to the young group, and the S(f) evolution in spectrogram characteristics with accumulation of fatigue is mostly absent. As shown in Figure 10a, S(f) only increases from Beginning to End are only observed in the [5-25 Hz] band (70% increase; Student-t test; t = 3.51; p = 0.01), reflecting reduced response of back muscle activation in old subjects with accumulation of fatigue at intermediate time scales. These results are in agreement with previous research (49), where an inability to activate tibialis anterior from 30 to 60 Hz was found in old, compared to young adults. As the erector spinae, tibialis anterior is mainly composed by type I fibers (43). As explained in previous sections, lumbar muscle degeneration and the reduced force levels in the elderly (54) might be responsible for these results.

Back muscle spectral power response to residual fatigue. A very similar spectral profile for the back muscle (erector spinae, ES) is observed in Exercise 3 (Fig. 8b), compared to Exercise 1 (Fig. 8a). However, starting spectral power at the Beginning of Exercise 3 is higher compared to Exercise 1 for low frequencies [5-25 Hz] (100% increase) and intermediate frequencies [25-50 Hz] (60% increase) in Regime 1, due to residual fatigue from previous exercise segments. Accordingly, ES-R spectral power increase from Beginning to End of Exercise 3 is less pronounced than in Exercise 1 (80% increase in the low [5-25 Hz] and 40% in the intermediate [25-50 Hz] bands in Regime 1; Wilcoxon matched pairs test; Z = 2.73; p = 0.006 and Z = 2.01; p = 0.004) (Fig. 8b). No changes are observed in the high [50-150 Hz] band in Regime 2.

Regarding the old group (Fig. 10b), the 65% elevation in spectral power of Regime 1 due to residual fatigue at the Beginning of Exercise 3, compared to the Beginning of Exercise 1, is less pronounced than for the young subjects (100% increase; right panel in Fig. 8b). Furthermore, similarly to Exercise 1, there is a markedly different response of Regime 1 and Regime 2 to accumulation of fatigue during Exercise 3 in the old group. However, the increase in Regime 1 power during Exercise 3 is less pronounced compared to Exercise 1 (50% in the [5-25 Hz] p = 0.04; and 20% in the [25-50 Hz] bands; right panels in a and b), indicating reduced back muscle response to accumulation of fatigue during Exercise 3 due to the existing residual fatigue from Exercise 1 and 2.

Notably, the evolution of the ES-R spectral profile with accumulated fatigue at intermediate time scales within an exercise (Figs. 6, 8, and 10), is remarkably consistent with the observed spectral power evolution at large time scales of consecutive exercise segments (Figure 5b). Specifically, the changes on the ES-R power are mainly concentrated in the low [5-25 Hz] and intermediate [25-50 Hz] bands in Region 1, while the evolution of high frequency [50-150 Hz] band in Regime 2 is completely absent.

3.5. Leg- and back-muscle myoelectrical activity and spectral power profiles at short time scales of a single squat movement

In previous sections we demonstrate that the leg and back spectral profiles in both young and old groups are robust as are consistently reproduced at large (Fig. 4 and Fig. 5) and intermediate time scales (Fig. 6 to Fig. 10). Finally, we test the robustness of such spectral profiles at short time scales of a single squat movement and their response to accumulated fatigue during Exercise 1 (Fig. 11 to Fig. 14). Specifically, we identify the leg and back spectral profiles for both Down and Up squat phases, characterized by different types of muscle contractions (lengthening and shortening contractions, respectively).

Leg muscle activation and spectral power evolution during Down and Up squat phases. While at the Beginning of Exercise 1 the EMG amplitude of the leg muscle (vastus lateralis, VL-R) clearly increases with transition from Down to Up phase within a squat leading to a bimodal profile, with progression of exercise the amplitude in both squat phases increases due to fatigue accumulation, and the transition from Down to Up phase in each squat becomes less pronounced (Fig. 11a, top panels). As at intermediate and large time scales, the VL-R spectral profile in young subjects is characterized by two distinct frequency regimes – Regime 1 of low [5-25 Hz] and intermediate frequencies [25-50 Hz] bands, and Regime 2 of high frequencies [50-150 Hz] band – with higher concentration of spectral power in Regime 1 during the Down phase and larger increase of spectral power in Regime 1 compared to Regime 2 in the Up phase of the squat (Fig. 11a, bottom panels). Specifically, spectral power significantly increases from Down to Up at the Beginning of exercise, for both low and intermediate frequencies in Regime 1 (Wilcoxon matched pairs test; $Z = 3.18$; $p = 0.001$), and high frequencies in Regime 2 ($Z = 3.18$; $Z = 0.001$) (Fig. 13a). A feasible interpretation for the overall higher spectral power in the Up compared to Down phase may be related to two different aspects. First, as described by in a previoys detailed review (18), EMG amplitude during lengthening contractions is lower than during shortening contractions. This reduction is due to a greater force capacity of muscle during lengthening contractions, which in turn leads to a diminished motor unit recruitment and discharge rate. Second, the higher spectral power observed in Up could also be influenced by the existing stretch-shorten cycle in the transition from Down to Up, that is, the increase in muscle length followed immediately by a shortening of the muscle (18,66).

The characteristic VL-R spectral profile and its Down-Up phase transition observed in the young group is robust as it is also present for consecutive squat movements, although with increased total power in response to accumulated fatigue at the End of Exercise 1 (Fig. 11a, bottom panels). Note that this Down-Up increment in spectral power is always higher at the Beginning compared to the End of exercise (Fig. 13a, red arrows). The reduced Down phase spectral power due to the typical reduction in neuromuscular activity associated with lengthening contractions (18), may be responsible for the higher increment in the spectral power from the Down to Up phase at the Beginning (i.e., no fatigue condition) compared to the End of exercise. The Down-Up spectral power increment at the End of exercise (Fig. 13a, red arrows) is lower given the high level of accumulated fatigue, which provokes elevated myoelectrical activity not only in the Up, but also in the Down squat phase. Accordingly, young subjects exhibit a significantly higher ratio of the Down phase spectral power at the End vs. Beginning of exercise ($<S(f)>_{End}/S(f)>_{Begin}$) compared to the same ratio for the Up phase (Fig. 13c; Wilcoxon matched pairs test comparing End vs Beginning of exercise for the Down squat phase vs. the Up squat phase gives $Z = 2.20$: $p < 0.02$).

As for the old group (Fig. 11a, bottom panels), similar spectral profiles with transition from Down-Up phase for a single squat and similar response to accumulated fatigue, are observed for the VL-R compared to young subjects (Fig. 12a, bottom panels), however, with reduced change in the Down phase spectral power from Beginning to End (300% and 80% increase in Down-phase power from Beginning to End of exercise in young and old subjects, respectively). As a result, the Down phase ratio ($<S(f)>_{End}/S(f)>_{Begin}$) in the old group is close to 1 for all the frequency bands (Fig. 13c) and. A feasible explanation for the reduced Down phase spectral power evolution during exercise, may be related to the preservation of eccentric muscle strength in older adults, due to the non-contractile and structural properties intrinsic to the muscle. More specifically, the accumulation of connective tissue within the muscles with age increases passive stiffness, which might offer a mechanical advantage during lengthening contractions (55,71). In turn, such aging effect could have also affected VL-R activation in the Down squat phase, leading to decreased Down spectral power evolution during exercise.

Back muscle activation and spectral power evolution during Down and Up squat phases. In contrast to the bimodal EMG amplitude profile of the leg (VL-R) muscle, myoelectrical activity of the right back erector spinae (ES-R) muscle exhibits a unimodal EMG amplitude profile, with no Down-Up transition in squats (Fig. 11b, top panels). The spectral profile of the ES-R is characterized with two distinct frequency regimes with a similar effect of fatigue accumulation, represented by an elevated total power at the End of exercise for both the Down and Up phases (Fig. 11b, bottom panels). However, in contrast to the leg muscle, the back muscle spectral power does not increase with transition from the Down to Up phase of the squat for any frequency band, neither at the Beginning nor at the End of exercise (Fig. 14a). The lack of Down-Up transition in the back spectral power, as well as the smaller rate of increase in the total power with accumulation of fatigue at the End of exercise, reflect the different role ES plays during the squat compared to VL. While force generation is the primary function of the VL, the main role of the back muscle is trunk stabilization (83).

Regarding the old group (Fig. 12b), similar spectral profiles with two frequency regimes and no transition from Down-Up phase within a single squat are observed as for the young subjects. However, note the dramatic decline (almost 1 decade) of a single squat total spectral power of the ES-R in old subjects compared to the young. Similar to the VL-R Down phase for the old group (Fig. 13b), the ES-R Down spectral power does not increase from the Beginning to End of exercise with accumulation of fatigue for any frequency band (Fig. 14b). Accordingly, the Down phase ratio (i.e., $S(f)_{End}/S(f)_{Begin}$) is about 1 for all the frequency bands in old subjects (Fig. 14c), indicating reduced back muscle response during lengthening contractions.

Notably, the leg and back muscle spectral profiles and its evolution observed at short time scales during a single squat movement, are also observed at intermediate time scales during separate exercise segments (Figs. 7, 8, 9, and 10) and at large time scales of consecutive exercise segments (Fig. 4c,d and Fig. 5b).

4. Discussion

The present study investigates the leg and back muscle spectral power profiles, and their evolution during three consecutive extended squat tests performed until exhaustion and four interspersed rest periods, over a range of large, intermediate, and short time scales – across repeated exercise and rest segments; within exercise segments; and during a single squat movement. In summary, (1) we identify the spectral profiles for both leg and back muscle activation with their muscle-specific evolution in response to fatigue according to the distinct histochemical properties and role of each muscle during the squat movement, (2) both low- and high-frequency regimes within the spectral profiles of leg and back muscle exhibit a muscle-specific evolution path, (3) leg and back muscle spectral power profiles reveal similar characteristics over a range of time scales, and (4) young and old subjects exhibit similar form of the spectral profiles for leg and back muscle activation, however, old subjects are characterized by different evolution path with less pronounced increase of spectral power in response to exercise and fatigue. The observed spectral power profiles for the leg and back muscle show universality as they are consistently reproduced for subjects in each age group (young or old), for each muscle type (leg or back) at a given physiological state (rest or exercise), and are robust since the general form of the profiles is preserved across exercise segments. Our approach offers new insights into a previously unrecognized mechanism of multi-scale organization and integration of different frequency bands, that represents coordinated activity among distinct types of muscle fibers in generating global muscle tone and its modulation in response to exercise-induced fatigue and aging.

We uncover a particular spectral profile for both leg VL muscle and back ES muscle and how it changes in response to accumulated fatigue during exercise segments and to residual fatigue for consecutive exercise segments. Specifically, we show the following differences between muscles: (a) with increasing fatigue the VL spectral power increases for both low and intermediate frequencies in Regime 1 (below 50 Hz), and for high frequencies in Regime 2 (above 50 Hz), while in contrast the ES spectral power mainly increases for low and intermediate frequencies in Regime 1 (below 50 Hz); (b) an overall reduced spectral response to fatigue is observed for the ES compared to the VL muscle; and (c) higher spectral power in the Up phase of each squat compared to the Down squat phase is observed for the VL, while in contrast no differences are observed between the Up and Down squat phases for the ES muscle. The reported differences in the spectral profile of the leg and back muscle activation and the differentiated response of different frequency bands to exercise and fatigue of these two muscle types may result from muscle-specific histochemical properties (muscle fiber content) and the distinct role during the squat movement of the VL and ES muscles. According to their fatigue and contractile characteristics, motor units are classified into three major types: slow fatigue-resistant, fast fatigue-resistant, and fast fatigable motor units, which typically consist of type I, type IIA and type IIX muscle fibers (7, 8, 63, 87). While the back ES muscle is mainly composed of type I slow/oxidative fibers (9,79), the leg VL muscle contains a higher percentage of type II fast/glycolytic fibers (64,83). Furthermore, although both leg and back muscles show high myoelectrical activity during squats, their role during the movement is different – while force generation is the primary function of VL, the main role of the ES muscle is trunk stabilization (83).

Our findings demonstrate that the different muscle fibers composing a certain muscle show a universal spectral profile and evolution in response to exercise-induced fatigue, as it can be robustly observed over a range from large time scales (i.e., within and across exercise segments; minutes to hours) to short time scales (during a single squat movement; seconds) in both young and old subjects. Human physiology presents a remarkable amount of distinct rhythms at all organismic levels (61), that are coupled and coordinated with each other over several magnitudes of time scales (30, 37, 40, 41, 44) to generate integrated physiologic functions associated with different systems (1, 39, 51). Exercise-induced physiological adaptations evolve as a consequence of soft-assembled states dwelling on different time scales and levels of biological systems organization (36). For instance, while at kinematic level exercise-related adaptations dwell at short time scales of seconds to minutes (35), at a performance level the changes occur at scales of days, weeks, and months. Thus, further research is warranted to investigate the changes in muscle spectral profiles at much larger time scales (weeks or months) as a consequence of a training intervention.

Our approach reveals a universal spectral power behavior across subjects and age groups in response exercise-induced fatigue. However, the following differences between age groups should be highlighted: (a) overall reduced spectral power evolution in the high frequencies Regime 2 (above 50 Hz) for old subjects, and (b) less pronounced spectral power evolution for the back ES muscle in old subjects across and within exercise segments compared to the young group. A feasible explanation for these divergences may stem from the reduction in the discharge rate of motor neurons in old adults (48), and from sarcopenia related normal decline of skeletal muscle and strength during aging (57, 91). Sarcopenia involves primarily type II muscle fibers, which fire above 50 Hz (28,87). The reduced back ES muscle spectral power evolution in the old group, might be related to the reported here absence of ES spectral power changes during the Down squat phase (i.e., lengthening contractions) with the course of exercise. Such effect could be explained by the accumulation of intramuscular connective tissue with aging, which significantly increases passive stiffness (71). The lack of spectral power evolution of the back ES muscle during the Down squat phase with accumulation of fatigue is a physiologically relevant finding, since lengthening contractions appear to be of key importance for the absorption of kinetic forces during the descent phase of a fall impact in old individuals, which could decrease the risk of hip fracture (74).

Given that sarcopenia mainly affects type II muscle fibers, and since the leg VL contains higher proportion of type II fibers than the back ES muscle, one would expect larger effect of aging for the leg VL muscle. However, our results show the opposite, namely, a more pronounced aging effect for the ES muscle. Our findings demonstrate that the mechanisms underlying muscular changes during aging are specific to individual muscle types, as also indicated in earlier works on human limb and trunk muscles (19, 59). Furthermore, the reduced ES spectral power could have been provoked by other reasons aside from sarcopenia in type II muscle fibers. We note that the cause of decreased back muscle function with aging can also be attributed to the reduction in quality and quantity of muscle excitability and contractility (52, 77, 82), which are in turn related to changes in the volume of intra-muscular adipose tissues, histological composition, and motor neuron signaling with aging (27). Accordingly, a pronounced decline in back muscle strength is typically observed after the sixth decade of life (20).

The presented here experimental and analytic framework with focus on quantifying the spectral power profile of different muscle types and its evolution during exercise to investigate the multi-scale mechanism underlying the differentiated response of distinct frequency bands to fatigue accumulation provides physiologically relevant information and new insights on how different muscle fibers, composing the skeletal muscle, respond to exercise-induced fatigue and aging. From a practical point of view, our approach can be utilized to assess and quantify the efficiency of training programs. Since spectral power profiles of muscle activation and their response to exercise-induced fatigue are specific for each muscle type, tracking changes in the spectral profiles alongside other physiological markers could help quantify more precisely physiological adaptations after exercise and training interventions, and may assist coaches with selection of the most appropriate training programs. For instance, the lack of changes in a given frequency band for a certain muscle after a training period, could indicate that the previous intervention was not effective to provoke adaptations in the corresponding muscle fibers. This might be particularly relevant for exercise and training programs targeting specific muscle fibers in elderly subjects (47), cancer patients (31), or patients with neurodegenerative disorders (45), where the activation and training of type-II muscle fibers is very important in preventing muscle atrophy and strength degradation. Further research is needed to (i) confirm universality of results over larger cohorts of subjects; (ii) investigate muscle spectral profiles under different clinical conditions (e.g., acute and chronic muscle injuries, neuro-muscular disorders etc), and (iii) identify muscle spectral power responses to distinct types of training programs (e.g., resistance, endurance).

The reported here findings should be considered in the context of certain limitations, including the limited number of subjects in both young and old group. Nevertheless, since participants performed three consecutive squat tests, each with duration of 6-12 min, the utilized protocol provides a sufficient number of squat movements necessary to perform reliable analyses of the spectral power profiles and to assess effects of accumulated and residual fatigue (Results Section 3.1). Due to the limited number subjects in each age group, we are unable to study gender effects. Further, we note that the magnitude of EMG signals may be influenced by variations in skin thickness and subcutaneous tissues among subjects. Given that elderly subjects present higher body fat mass compared to young adults (70), the observed differences across age groups might be partially influenced by different body fat levels in the two groups. However, as described in earlier studies (77), this artifact has been shown to mainly affect the EMG signal amplitude, and has relatively little effect on the functional form of the signal spectral power distribution in the frequency domain. Lastly, the present study is not supported by kinematic data, and ankle, knee, and hip angles are not examined throughout the Down and Up squat phases.

5. Conclusions

In summary, we investigate leg and back muscle activation during repeated maximal exercise. We find that leg and back muscle dynamics are characterized by muscle-specific spectral power profiles, where different frequency bands exhibit a differentiated response to accumulation of fatigue within exercise segments and to residual fatigue across repeated exercise segments. The established universality in general spectral power profile characteristics among subjects in both young and old groups indicates presence of a previously unrecognized multi-scale mechanism underlying the distinct response of different muscle types to exercise-induced fatigue and effects of aging. The consistency of the spectral power profiles among subjects (young or old group), at a given physiological state (rest or exercise) and muscle type (leg or back), as well as the robustness in the evolution of these profiles at large time scales for consecutive exercise segments (hours), intermediate time scales within exercise segments (minutes), and at short time scales during a single squat movement (seconds), reveals a universal behavior related to a basic multi-scale mechanism of muscle tone regulation that integrates the activation of different muscle fibers types across frequency bands. To our knowledge, this is the first research uncovering differentiated response and evolution across time scales of the leg and back spectral power profiles in healthy young and old adults with accumulation of fatigue during exercise.

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Disclosures

The authors declare that they have no conflict of interest.

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7. Figure captions

Fig. 1 Leg and back muscle myoelectrical activity during exercise at different time scales. Evolution of myoelectrical activity represented by EMG amplitude profiles at a) large time scale of consecutive exercise segments and b) short time scale of individual and consecutive squats, comparing the right and left leg vastus lateralis muscle (VL-R, VL-L) with the right and left back erector spinae (ES-R, ES-L), for a typical young subject. The exercise protocol is composed by three consecutive squat tests, each performed until exhaustion, separated by 10min rest periods in supine position; EMG data is recorded with 500 Hz sampling frequency. Leg and back muscles show different EMG amplitude profiles Auat both a) large at b) short time scales. a) Within and across exercise segments the leg EMG amplitude gradually increases reflecting the effect of fatigue, in contrast to the back muscle where the EMG amplitude does not change significantly. Note also the higher initial EMG amplitude at the Beginning of Exercise 2 and 3 compared to Exercise 1 for the leg muscle indicating residual fatigue – an effect that is not present for the back muscle. b) Markedly different EMG amplitude profiles are observed for the leg and back muscles at short time scales of a few seconds, associated with individual squats – bimodal profile for the leg muscle with two phases corresponding to the down (smaller amplitudes) and up (larger amplitude) squat movements. The observed differences between the leg and back muscles in the EMG amplitude profiles at both short and large time scales and the profiles evolution with fatigue reflect different muscle fiber structure and role to squat movement. These empirical observations motivate our hypothesis that distinct muscles have specific spectral power profiles, with different role of muscle fibers to the spectral power of high- and low- frequency components, and muscle-specific trajectory for the evolution of spectral profiles across short and long time scales in response to exercise-induced fatigue and aging

Fig. 2 Leg and back muscles spectral power density profiles and their evolution across rest and exercise segments in young subjects. Group average spectral power S(f) distribution curves for the right leg vastus lateralis muscle (VL-R) and right back erector spinae muscle (ES-R) for consecutive rest (a,b) and exercise (c,d) segments of squat tests (Methods section). While for the resting periods leg and back muscle exhibit similar spectral behavior, their EMG dynamics during exercise are characterized by different S(f) curve profiles, indicating a clear relationship between muscle type and spectral power. This relationship is consistently present for all individual subjects in the group (not shown), demonstrating that each muscle type is characterized by a robust spectral power distribution shape. A significant increase in S(f) is observed with accumulation of fatigue from Exercise 1 to Exercise 2 for both and leg and back muscle, however, preserving the muscle specifc profile of S(f). Note, that the leg muscle response to exercise and fatigue is quantified by spectral power is with a factor of 2 higher compared to the back muscle. Spectral power is obtained after applying a 50 Hz notch filter to EMG signals to remove power grid line interference, and was computed for each muscle type and exercise-rest segment, in 2 sec moving window with 1 sec overlap. Results for the left leg (VL-L) and left back (ES-L) muscle are consistent with the young group averaged results shown for the right leg (VL-R) and right back muscle (ES-R) in Fig. 2

Fig. 3 Leg and back muscles spectral power density profiles and their evolution across rest and exercise segments in old subjects. Group average spectral power S(f) distribution curves for the right leg vastus lateralis muscle (VL-R) and right back erector spinae muscle (ES-R) for consecutive rest (a,b) and exercise (c,d) segments of squat tests (Methods section). For the leg muscle, a similar response to (b) rest and (d) exercise, with similar spectral power evolution with accumulated fatigue, is observed in the old subjects compared to the young group shown Fig. 2. In contrast, the back muscle exhibits a much reduced spectral response to (b) rest and (d) exercise and fatigue in old subjects compared to the young group. EMG signal pre-processing, spectral power calculation, and group averaging are performed as in Fig. 2 (Methods section). Results for the left leg (VL-L) and left back (ES-L) muscle are consistent with the old group averaged results shown for the right leg (VL-R) and right back muscle (ES-R) in Fig. 2

Fig. 4 Leg and back muscles total spectral power for rest and exercise in young and old subjects. Barcharts representing group averaged total spectral power (i.e., area under the S(f) curves in Figs. 2 and 3) for (a,c) right leg vastus lateralis muscle (VL-R) and (b,d) right back erector spinae muscle (ES-R) for consecutive rest and exercise segments. With transition from rest to exercise, both leg and back muscles total spectral power increase 10^{-2} - 10^{-3} times, in young and old subjects. (a,c) No significant differences are observed for the total spectral power of the leg muscle activation between young and old subjects, for both rest and exercise. (b,d) In contrast to the leg muscle, the total power of back muscle is significantly higher in young subjects, for both rest and exercise (comparing young vs. old: Student t-test $p < 0.02$ for rest periods and $p < 0.01$ for exercise periods). Leg muscle activation in both age groups shows significant effect of accumulated fatigue with (c) 36% increase in spectral power for young subjects and 40% increase for old subjects from Exercise 1 to 2 (ANOVA repeated measures test $p < 0.01$ for both groups). In contrast, the back muscle exhibits significant evolution in spectral power across exercise segments with (d) 23% increase from Exercise 1 to Exercise 2, only for the young subjects (ANOVA repeated measures $p = 0.04$ for young; $p = 0.07$ for old). Error bars indicate standard error. Results for the left leg (VL-L) and left back (ES-L) muscles are consistent with the results shown for the right leg (VL-R) and right back (ES-R) muscles shown in the figure

Fig. 5 Frequency bands contribution to the spectral power profile and its evolution at large time scales of consecutive exercise and rest segments. Spectral profiles show distinct contributions of different frequency bands to the total power of leg (vastus lateralis, VL-R) and back (erector spinae, ES-R) muscles for (a) rest and (b) exercise. Myoelectrical activity exhibits markedly different spectral profiles for rest vs. exercise: (a) at rest the low frequency [5-25Hz] band is the only contribution to total spectral power; in contrast, (b) for exercise the low [5-25Hz] and intermediate [25-50Hz] frequency bands dominate the spectral power, with significant contribution from the high frequency [50-150Hz] band. The observed differences between rest and exercise, which are present for both leg and back muscles, indicate that each physiological state is characterized by a specific spectral profile. Significant reduction in spectral contribution from the intermediate [25-50Hz] frequency band compared to the low frequency [5-25Hz] band in back muscle for consecutive exercise segments (Student t-test $p < 0.002$) which is not observed for the leg muscle (Student t-test $p > 0.2$), demonstrate muscle differentiate response to exercise and fatigue accumulation. For consecutive exercise segments, spectral power increases (with approximately 30%) proportionally for all frequency bands, in both young and old groups, reflecting common response across the entire frequency range to accumulated fatigue. Results for the left leg (VL-L) and left back (ES-L) muscles are consistent with the results shown for the right leg (VL-R) and right back (ES-R) muscles shown in the figure. The consistency of spectral profiles among subjects from a given group (young - old) at a given physiological state (rest - exercise), and the robustness of these profiles for repeated rest and exercise segments indicate a universality behavior related to a basic mechanism of muscle tone regulation. Results for the left leg (VL-L) and left back (ES-L) muscle are consistent with the results shown for the right leg (VL-R) and right back (ES-R) muscle shown in the panel

Fig. 6 Evolution of spectral power at intermediate time scales in the course of exercise. Spectrograms of EMG signals (500 Hz sampling frequency) obtained from the right leg (vastus lateralis, VL-R) and the right back (erector spinae, ES-R) muscles of a representative young an old subject, during a single exercise segment (approximately 10 min of a squat test performed until exhaustion. Spectral power is calculated in moving time windows of 2 sec with 1 sec overlap, over the entire duration of the exercise segment (x axis), and in frequency bands of 10 Hz (y axis) over the entire range of physiologically relevant frequencies [5-200 Hz] (y axis). Spectrograms are colored coded with warm colors, indicating periods of high spectral power. With progression of the exercise, there is a pronounced increase both in spectral power level and in the range of active frequency bands, in response to effort and accumulation of fatigue – a clear evolution in the spectral profile from fewer active frequency bands with relatively lower spectral power at the Beginning of exercise to a broader range of frequency bands activated at higher level when approaching exhaustion at the End of the exercise . Notably, these fatigue effects are more pronounced for the leg compared to the back muscle (a, top panels) – back muscle spectral power in high frequency bands (above 55 Hz) does not increase in the course of exercise, and response to fatigue with widening range of active frequency bands and increased power is observed only for low and intermediate frequencies. In old subjects, the evolution in spectrogram characteristics with accumulation of fatigue is less pronounced for the leg muscle, (a, bottom panels) and is not present for the back muscle. The differences between the leg and back muscle spectral profiles and their evolution with fatigue at intermediate time scales during exercise are consistent with the behavior at large time scales where back muscle total spectral power remains unchanged for repeated exercise segments in Figure 4. Spectrograms obtained for the left leg (VL-L) and left back (ES-L) muscles (not shown) are consistent with the results for the right leg (VL-R) and right back (ES-R) muscles across all the subjects in the young and old group

Fig. 7 Leg-muscle spectral profile, its evolution with accumulated fatigue at intermediate time scales during exercise, and response to residual fatigue at large time scales across consecutive exercise segments. Detailed spectral power distribution profiles of right leg (vastus lateralis, VL-R) muscle activation presented in frequency bands of 4 Hz for periods of 1 min (10 squats) in the Beginning and End of (a) first and (b) third exercise segment (see protocol in Methods section). Profiles represent S(f) group average, and error bars show standard error for group average power in every 4 Hz bin. Yellow stars in left panels indicate the frequency bands with statistically significant differences in spectral power between Beginning and End. Profiles are characterized by two separate Regimes with different response to accumulating fatigue in the course of exercise (a, left panel) – Regime 1 corresponding to low [5-25 Hz] and intermediate [25-50 Hz] frequency bands with dramatic 200% in spectral power (Wilcoxon matched pairs test $p < 0.003$) at the end of the exercise, and a Regime 2 including high frequency bands [50-150 Hz] with less pronounced increase (~100%; Wilcoxon matched pairs test $p < 0.001$) in spectral power (a, right panel). Similar S(f) profile with two frequency Regimes exhibiting distinct response to accumulated fatigue is observed also during Exercise 3 (b, left panels), however, with much higher 100% starting total spectral power compared to the start of Exercise 1, reduced response (~50% increase; Wilcoxon matched pairs test $p < 0.002$) in low [25-50 Hz] and intermediate [25-50 Hz] bands, and no change in the power of high frequencies [50-150 Hz] (b, right panel) – all due to residual fatigue from previous exercise segments. The observed evolution in the S(f) profile from the Beginning to the end of an exercise and with consecutive exercise segments, reflects a differentiated role of leg muscle fibers with different contribution in response to exercise-induced fatigue. Note that the observed evolution in the spectral profile of the leg muscle at intermediate time scales within an exercise in response to accumulated fatigue (shown here) is consistent with the results shown for the change in spectral power in the leg spectral power at large time scales of consecutive exercise segments (Figure 5b) where the power of all the frequency bands increases from Exercise 1 to Exercise 2 but does not change from Exercise 2 Exercise 3 due to effects residual fatigue.

Fig. 8 Back-muscle spectral profile, its evolution with accumulated fatigue at intermediate time scales during exercise, and response to residual fatigue at large time scales across consecutive exercise segments. Detailed spectral power distribution profiles of right back (erector spinae, ES-R) muscle activation presented in frequency bands of 4 Hz for periods of 1 min (10 squats) in the Beginning and end of (a) Exercise 1 and (b) Exercise 3 segments (see protocol in Methods section). Profiles are obtained following the same procedure as in Figure 7. Similar to leg muscle activation, the back spectral power also increases with fatigue accumulation during exercise (a, left panel), an effect which is less pronounced compared to the leg muscle and concentrated only in Regime 1 – 140% increase in S(f) in the low [5-25 Hz] band and 70% in the intermediate [25-50 Hz] band (Wilcoxon matched pairs test $p > 0.02$) (a, right panel). This evolution in S(f) profile in the course of exercise is consistently observed also for Exercise 3 (b, left panel), although with higher starting spectral power at Beginning of Exercise compared to Exercise 1, with less pronounced effect in Regime 1 (b, right panel), due to residual fatigue from previous exercise segments. Episodes of response to fatigue in the high frequencies [50-150 Hz] Regime 2 of the back muscle spectral profile reflect different muscle fiber composition and their different contribution to squat movement compared to the leg muscle. Notably, the evolution of the back muscle spectral profile with accumulated fatigue at intermediate time scales within an exercise (shown here) is remarkably consistent with observed spectral power evolution at large time scales of consecutive exercise segments (Figure 5b) where the power of low and intermediate frequencies significantly increases while high frequency (bigger than 50 Hz) contribution to spectral power remains unchanged. Yellow stars in left panels indicate the frequency bands with statistically significant differences in spectral power between Beginning and End.

Fig. 9 Effects of aging on leg-muscle spectral power response to accumulated fatigue at intermediate time scales during exercise and to residual fatigue at large time scales across consecutive exercise segments. The results are obtained following the same experimental protocol, data processing and analysis procedure, and statistical tests as shown for the group of young subjects in Figure 7 (Methods section). Error bars indicate standard error. Yellow stars in left panels indicate the frequency bands with statistically significant differences in spectral power between Beginning and End. Detailed analysis of the right leg muscle (vastus lateralis, VL-R) spectral power contribution of different frequency bands in old subjects shows a very similar shape of the S(f) profile as well as similar profile evolution from Beginning to end of the exercise for individual subjects and their group average in both young (Fig.7a) and old (a) groups. The spectral power profile S(f) exhibits two distinct regimes of frequency bands with (i) different starting levels of power at the Beginning of exercise, with higher power for Regime 1 of low [5-25 Hz] and intermediate [25-50 Hz] frequencies, and lower power for Regime 2 of high [50-150 Hz] frequencies); and (ii) distinct evolution of frequency regimes in response to accumulation of fatigue with progression of exercise, with more pronounced increase in power for Regime 1 compared to Regime 2. The observed leg muscle spectral power profile and its evolution with fatigue in the old group (a) is consistent with the leg muscle spectral response of young subjects in Fig. 7, indicating universality in myoelectrical activation during exercise. However, in contrast to the young group, the spectral power in Regime 1 of low and intermediate frequency bands in old subjects starts at significantly higher levels at the Beginning of Exercise 1 (with approximately 30%; Mann Whitney U test $p < 0.05$), indicating significantly elevated initial muscle tone after Rest 1 as an effect of aging. Note also that the spectral power increase in Regime 1 at the end of Exercise 1 is less pronounced (80% for [5-25 Hz] and 60% for [25-50 Hz] in old, vs. 240% and 170% respectively in young subjects), indicating a reduced response of the leg muscle activation in old subjects with accumulation of fatigue at intermediate time scales within an exercise. (b) Similar S(f) profile characteristics and evolution as in Exercise 1 are also observed for Exercise 3. Significantly elevated spectral power in Regimes 1 and 2 at the Beginning of Exercise 3 compared to Exercise 1 (Wilcoxon matched pairs test $p < 0.04$), with same level of elevation for the young (right panel, Fig. 7b) and the old group (right panel, b), indicates an effect of residual fatigue from Exercise 1 and 2 that is the same for both groups. This residual fatigue effect leads to a reduced response to accumulated fatigue during Exercise 3 compared to Exercise 1: less pronounced increase of 30% in the [5-25 Hz] and 20% in the [25-50 Hz] band in Exercise 3 compared to 80% and 60% correspondingly in Exercise 1 for old subjects (right panels in a and b), and approximately 50% increase for the [5-25 Hz] and [25-50 Hz] bands for Exercise 3 compared to 240% and 170% respectively for Exercise 1 in young subjects (right panels, Fig. 7a,b). Note also a similar level of power response in the Regime 1 frequency bands at the End of Exercise 3 and Exercise 1 corresponding to the maximum leg muscle capacity (all exercise segments are performed till full exhaustion, Methods section), which is significantly lower in old (right panels in a and b) compared to young subjects (right panels in Fig. 7a,b) (Mann Whitney U test $p < 0.04$). In contrast to Regime 1, there is no change in the spectral power of Regime 2 of high [50-150] frequencies. Such episode of increase evolution in Regime 2 during exercise 3 is in contrast to Regime 1, where there is a pronounced evolution resulting from Residual fatigue at the Beginning of Exercise 3. This effect is not seen for Exercise 1, where there is no effect of residual fatigue and where Regime 2 spectral power increases with accumulation of fatigue during the exercise. This behavior of Regime 2 in response to accumulated fatigue during exercise and in response to residual fatigue (Exercise 3) is consistently observed in both the young (Fig. 7b) and old groups

Fig. 10 Effects of aging on back-muscle spectral power response to accumulated fatigue at intermediate time scales during exercise and to residual fatigue at large time scales across consecutive exercise segments. Group average results for old subjects shown in the figure are obtained following the same experimental protocol, data processing, analysis procedure, and statistical tests as shown for the group of young subjects in Figure 8 (Methods section). Error bars indicate standard error. Yellow stars in left panels indicate the frequency bands with statistically significant differences in spectral power between Beginning and End. Detailed analysis of the contribution of different frequency bands to the right-back muscle (erector spinae, ES-R) spectral power shows S(f) profile shape and profile evolution during exercise that are consistently similar for individual subjects in both old (panels in a) and young (Fig. 8a) groups. The back muscle spectral power profile exhibits two distinct frequency regimes with: (i) different starting levels of power at the Beginning of exercise, where Regime 1 of low [5-25 Hz] and intermediate [25-50 Hz] frequencies has higher power (right panel in a) compared to Regime 2 of high [50-150 Hz] frequencies; and (ii) with different patterns of evolution in response to accumulation of fatigue with progression of exercise, where the power in Regime 1 significantly increases (Student t-test $p < 0.01$) while Regime 2 remains unchanged (Student t-test $p = 0.7$). The spectral power profile of the back muscle and its evolution with fatigue in the old group (panels in a) are consistent with the young subjects in Fig. 8, indicating universality in myoelectrical activation during exercise. Note that the spectral power increase in Regime 1 at the end of Exercise 1 is less pronounced in old subjects (70% increase for [5-25 Hz] and 30% for [25-50 Hz]; a, right panel; Student t-test $p < 0.01$) compared to young subjects (140% increase for [5-25 Hz] and 70% for [25-50 Hz]; Figure 8a, right panel; Wilcoxon matched pairs test $p < 0.002$), indicating reduced response of back muscle activation in old subjects with accumulation of fatigue at intermediate time scales within an exercise. (b) Spectral power profile characteristics with two distinct frequency regimes and their evolution with fatigue in Exercise 3 for the old group. S(f) behavior during Exercise 3 is similar to Exercise 1. Spectral power in Regime 1 at the Beginning of Exercise 3 is significantly higher compared to Exercise 1 (65% increase; Student t-test $p < 0.04$) due to residual fatigue from Exercise 1 and Exercise 2. Note that the 65% elevation in spectral power of Regime 1 due to residual fatigue at the Beginning of Exercise 3 compared to the Beginning of Exercise 1 is less pronounced for the old group than for the young subjects (100% increase; Student t-test $p < 0.04$; right panel in Fig. 8b), indicating reduced back muscle response to residual fatigue in old subjects. In contrast, the spectral power level in Regime 2 at the Beginning of Exercise 3 does not increase compared to the Beginning of Exercise 1, indicating differentiated response of frequency regimes to residual fatigue, that reflects specific back muscle fibers composition. As in Exercise 1, there is a markedly different response of Regime 1 and Regime 2 to accumulation of fatigue during Exercise 3 with significant increase in power of Regime 1 from Beginning to End of exercise (Student t-test $p < 0.05$) and no change in the power of Regime 2. However, the increase in Regime 1 power during Exercise 3 is less pronounced compared to Exercise 1

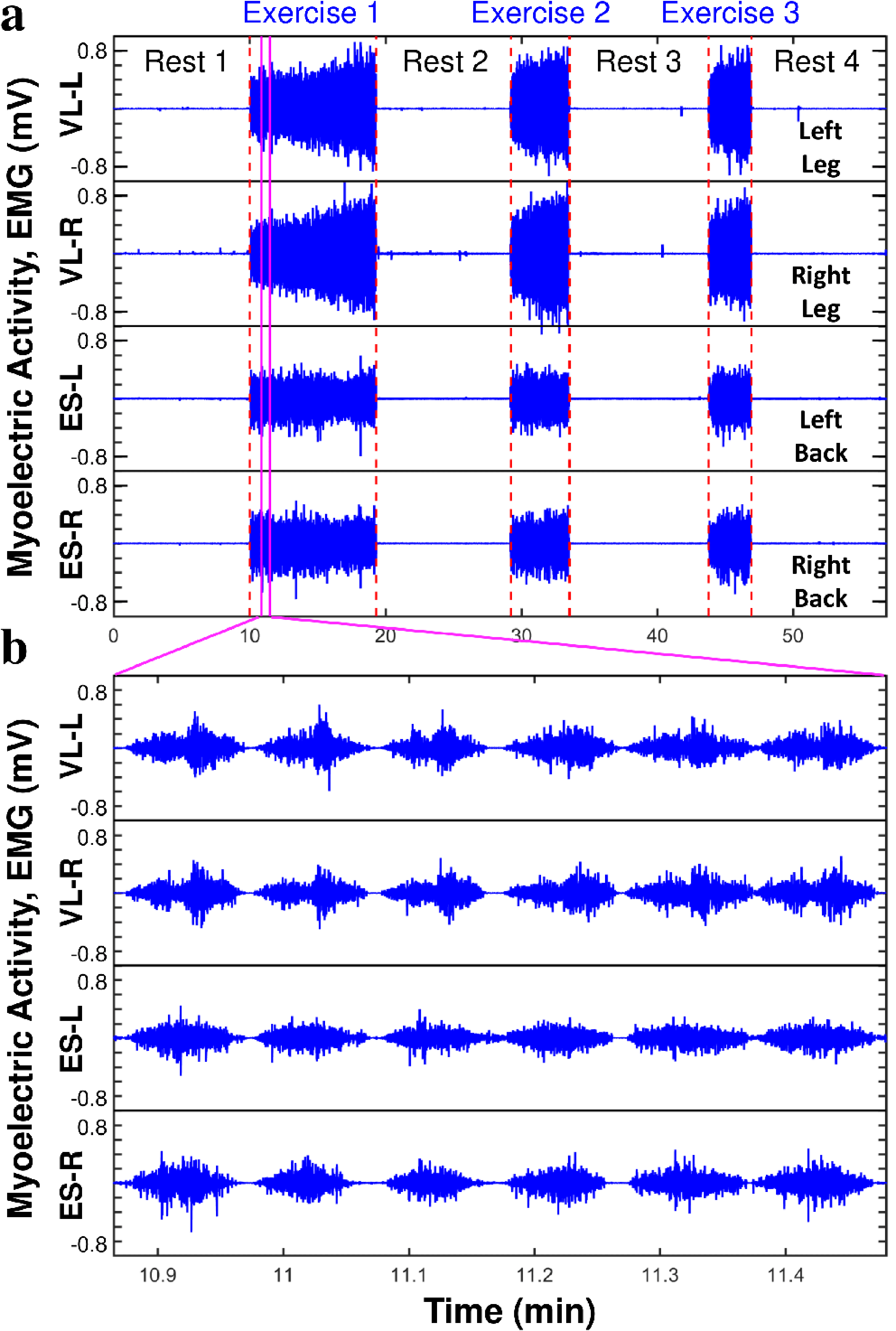
(50% in the [5-25 Hz] and 20% in the [25-50 Hz] bands in Exercise 3 compared to 70% and 30% correspondingly in Exercise 1 for old subjects; right panels in a and b), indicating reduced back muscle response to accumulation of fatigue during Exercise 3 due to the existing residual fatigue from Exercise 1 and 2. Same effect on spectral power response in Regime 1 (but with a larger amplitude) is also observed for young subjects with 80% ([5-25 Hz]) and 40% ([25-50 Hz]) increase in Exercise 3, compared to 140% and 70% respectively in Exercise 1 (right panels, Fig. 8a,b). In addition to this muscle type differentiation based on aging effect, the results in (a) and (b) show a remarkable dissociation in response to fatigue of muscle fiber within the back muscle as represented by different frequency bands: while there is a clear response to both accumulated and residual fatigue in Regime 1, there is no change in frequency Regime 2 (a behavior observed for both young and old subjects)

Fig. 11 Leg- and back-muscle profiles in myoelectrical activity and spectral power at short time scales of a single squat movement and response to accumulated fatigue during exercise. (a, top panels) Myoelectrical activity profiles of right leg muscle (vastus lateralis, VL-R) for the Down and Up phases of a single squat at the Beginning and End of Exercise 1 (Methods) for a representative young subject. While at the Beginning of exercise the EMG amplitude clearly increases with transition from Down to Up phase within a squat leading to a bimodal profile, with progression of exercise the amplitude in both squat phases increases due to fatigue accumulation and the transition from Down to Up phase in each squat becomes less pronounced. (a, bottom panels) Detailed leg muscle spectral profile $\widetilde{\boldsymbol{S}}(\boldsymbol{f})$ (presented in frequency bands of 4 Hz, Methods) and its evolution at short time scales from Down to Up phase within a squat at the Beginning (left column) and End (right column) of Exercise 1. Panels show the group average for all young subjects where the spectral profile of each subject is derived from 5 separate squat movements at the Beginning and End of Exercise 1 (Methods). Error bars in plots represent the group average standard error. As for intermediate and large time scales, the leg muscle spectral profile is characterized by two distinct frequency regimes – Regime 1 of low [5-25 Hz] and intermediate frequencies [25-50 Hz] bands and Regime 2 of high frequencies [50-150 Hz] band – with higher concentration of spectral power in Regime 1 during the Down phase and larger increase of spectral power in Regime 1 compared to Regime 2 in the Up phase of the squat (bottom panels in a, left column). This evolution in spectral power profile with Down-Up transition within a single squat, is consistently observed across subjects in the young group, and shows a differentiated response of distinct leg muscle fibers represented by different frequency bands, where slow muscle fibers (Regime 1) generates the dominant contribution to the spectral power increase in the Up phase, reflecting specific role of different muscle fibers during lengthening (Down phase) and shortening (Up phase) muscle contractions. This characteristic spectral profile and its Down-Up phase transition is robust as it is present for consecutive squat movements, although with increasing level of total spectral power in response to accumulated fatigue at the End of the exercise (bottom panels in a, right column). Notably, the leg muscle spectral profile and its evolution observed at short time scales within a single squat movement are also observed at intermediate time scales during separate exercise segments (Fig. 7 and Fig. 9) and at large time scales of consecutive exercise segments (Fig. 5b). (b, top panels) Myoelectrical activity of right back erector spinae (ES-R) exhibits an EMG amplitude profile that is unimodal with no Down-Up transition in squats at the Beginning and End of exercise – in contrast to the bimodal amplitude profile of the leg VL-R muscle (a, top panels). (b, bottom panels) Back muscle spectral power profiles for the Down and Up phase of squat movements at the Beginning of exercise, and spectral power evolution in response to fatigue at the End of exercise. Same protocol, data analysis, and group averaging procedures are performed as in (a). As the leg muscle in (a), the spectral profile of the back muscle is characterized with two distinct frequency regimes with a similar effect of fatigue accumulation, represented by an elevated level of total spectral power at the end of exercise. However in contrast to the leg muscle, the back muscle spectral power does not increase with transition from the Down to Up phase of the squat. Episodes of increase in the back muscle spectral power with Down-Up transition within each squat at both the Beginning and End of exercise, as well as the smaller rate of increase in total power with accumulation of fatigue at the End of exercise, reflect the secondary role back muscle plays in squat movements compared to leg muscle. Our findings for left leg (VL-L) and left back (ES-L) muscles (not shown here) are consistent with the results shown in (a) and (b)

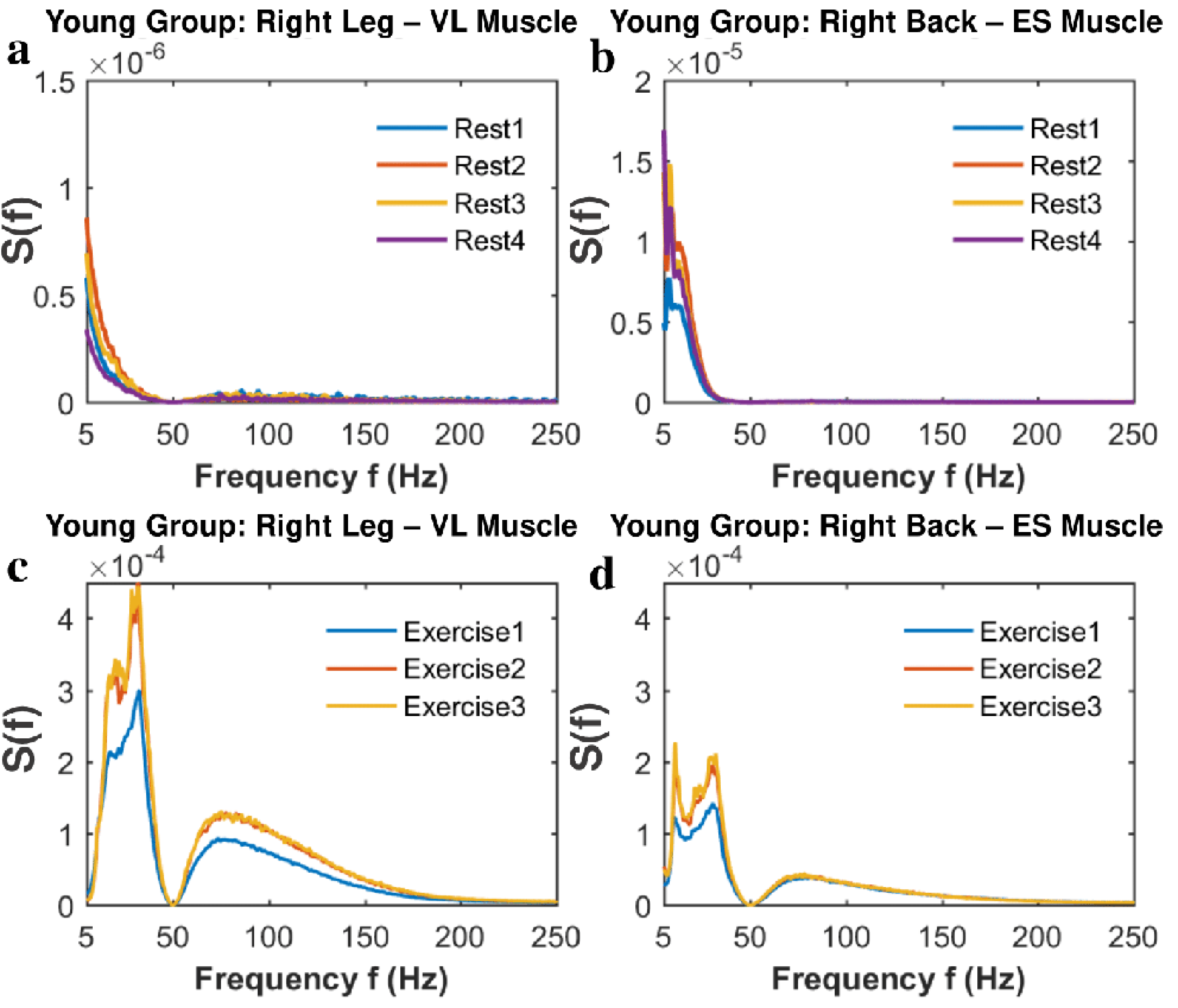
Fig. 12 Aging effects on leg- and back-muscle myoelectrical activity and evolution of spectral power profiles at short time scales of a single squat. Myoelectrical activity profiles of a single squat movement (Down and Up phase) of a representative old subject for the right leg muscle VL-R (a, top panels) and the right back muscle ES-R (b, top panels) at the Beginning and End of an exercise segment (Methods). The leg muscle myoelectrical activity during a single squat exhibits a bimodal profile, with lower EMG amplitude during the Down squat phase, transition to a higher amplitude during the Up phase, and significant increase in the EMG amplitude of the entire squat movement with progression of exercise due to accumulated fatigue – same general behavior as observed for the young group in Fig. 11. In contrast to the leg, the back muscle EMG squat profile is unimodal, with no difference in the EMG amplitude between the Down and Up squat phase, and less pronounced increase in the EMG amplitude with accumulation of fatigue at the End of exercise. Note, that these characteristics of the leg- and back-muscle EMG amplitude profiles during squat movements (i.e., bimodal vs. unimodal EMG profiles), and how the EMG profiles change with progression of exercise are consistently observed for both old (a and b, top panels) as well as for young subjects (Fig. 11), indicating strong association between the EMG amplitude profile and muscle type. The spectral power profile of the leg muscle (VL-R) for old subjects is characterized by two frequency regimes during both Down and Up squat phases, with a transition to higher spectral power during the Up phase (a, bottom panel) – consistent with the young group (Fig. 11). With progression of exercise, total power increases during both Down and Up phases, preserving the general shape of the spectral profile (a, bottom panels) – an effect of accumulation of fatigue which is less pronounced for the Down phase in old subjects (~80% increase in Down-phase power from Beginning to End of exercise; Wilcoxon matched pairs test $p < 0.05$) compared to young subjects (~300% increase in Down-phase power from Beginning to End of exercise; Wilcoxon matched pairs test $p < 0.01$; Fig. 11a), that can be related to increase in connective tissue in the muscle due to aging (see Results 3.5. for physiological interpretation). Similar to the leg VL-R muscle, the spectral profile of the back ES-R muscle exhibits two frequency regimes with higher concentration of power in Regime 1, however, without increase in spectral power with transition from Down to Up squat phase (b, bottom panel) – a behavior consistently observed also in young subjects for the back muscle (Fig. 11b). In contrast to the young group (Fig. 11b, bottom panels), the Down phase of the back-muscle spectral power in old subjects does not change from Beginning to End of exercise, indicating an aging effect of reduced back-muscle activation in response to fatigue that can be attributed to increased connective tissue in the muscle (same aging effect but less pronounced is present for the leg muscle; see bottom panels in (a) and Results 3.5.). Note the dramatic decline (~1 decade) in the back-muscle total spectral power of a single squat in old subjects compared to the young, indicating a reduction in back-muscle mass as a result of sarcopenia. Same protocol, data analysis, and group averaging procedures are performed for the old subjects in (a) and (b) subjects as for the young group in Fig. 11. Results for the left leg and left back muscle (not shown) are consistent with the results for the right leg and right back muscle shown in the figure

Fig. 13 Leg-muscle spectral power transition from the Down to Up phase of a single squat movement and response to accumulated fatigue during exercise. Right leg muscle (vastus lateralis, VL-R) spectral power transition from the Down to Up phase during a squat at the Beginning and End of Exercise 1 in young (a) and old subjects (b). Spectral power significantly increases from the Down to Up phase at the Beginning of exercise in young subjects (a), for low [5-25 Hz], intermediate [25-50 Hz], and high [50-150 Hz] bands (Wilcoxon matched pairs test $p < 0.05$), reflecting the presence of the stretch-shorten cycle – the increase in muscle length (Down phase) followed immediately by a shortening of the muscle (Up phase). The characteristic Down-Up transition in the leg-muscle spectral power is also present for consecutive squat movements, however, the Down-Up spectral power increase is less pronounced at the End compared to the Beginning of exercise (red arrows in panel a), due to the bigger increase in the Down-phase spectral power at the End of exercise compared to the spectral power increase in the Up phase (see Fig. 11a, bottom panels), indicating high level of accumulated fatigue. Accordingly, young subjects exhibit a significantly higher ratio of the Down phase spectral power at the End vs. Beginning of exercise compared to the same ratio for the Up phase (panel c; Wilcoxon matched pairs test comparing the End-Begin for the Down squat vs. the Up squat phase gives $p < 0.02$). As for the young group (a), the Down-Up spectral power transition is also present for old subjects (panel b, Wilcoxon matched pairs test gives $p < 0.05$ for all the frequency bands), however, with a reduced change in the Down phase spectral power from Beginning to End of exercise compared to young subjects (see Fig. 12a, bottom panels), reflecting reduced leg-muscle response during lengthening contractions with aging. As a result, the Down phase ratio in the old group is close to 1 for all the frequency bands (c). Error bars in plots represent the group average standard error. The red arrows indicate statistically significant changes in spectral power from Down to Up squat phase. Results for the left leg (not shown) are consistent with the results for the right leg muscle shown in the figure

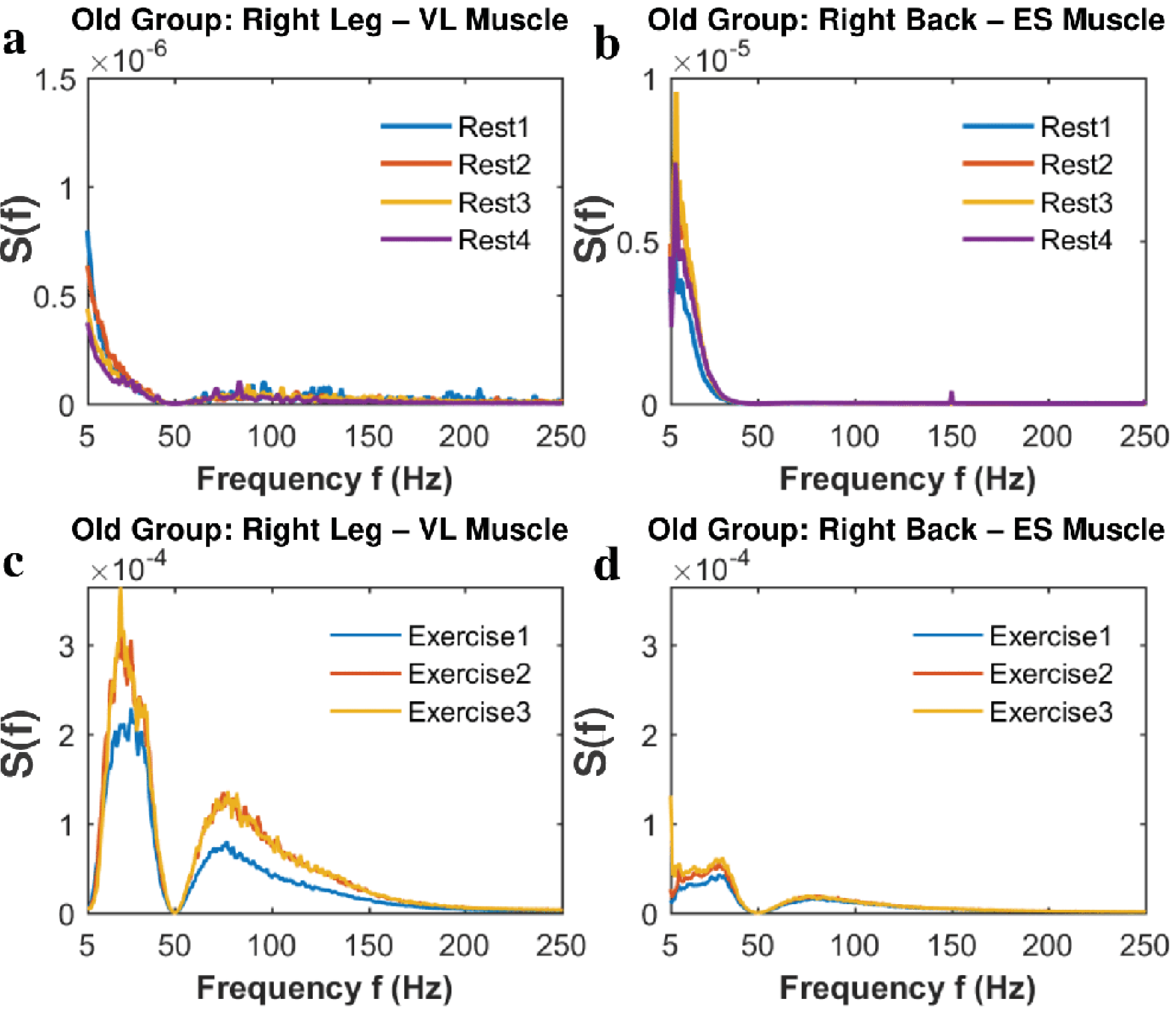
Fig. 14 Back-muscle spectral power transition from the Down to Up phase of a single squat movement and response to accumulated fatigue during exercise. Right back muscle (erector spinae, ES-R) spectral power transition from the Down and Up phase during a squat at the Beginning and End of Exercise 1 in young (a) and old subjects (b). In contrast to the leg muscle (Fig. 13), the back muscle spectral power for young subjects (a) does not increase with transition from the Down to Up phase for any frequency band, neither at the Beginning nor at the End of exercise, reflecting the different role back muscle plays during the squat compared to the leg muscle – while force generation is the primary function of the leg muscle, the main role of the back muscle is trunk stabilization. The back-muscle spectral power profile and its lack of Down-Up transition is also observed with accumulation of fatigue at the End of exercise, however, with an increased total power which is more pronounced in the Down than in the Up phase (see Fig. 11b, bottom panels). Accordingly, young subjects exhibit a higher ratio of the Down-phase spectral power at the End vs. Beginning of exercise compared to the same ratio for the Up phase (panel c; Wilcoxon matched pairs test comparing End-Begin ratio for the Down squat phase vs. the same ratio for the Up squat phase gives $p < 0.01$). As for the young group (a), back-muscle spectral power for old subjects (b) does not increase with transition from the Down to Up squat phase at the Beginning of exercise (Wilcoxon matched pairs test comparing Down vs. Up phase at the Beginning of exercise gives $p > 0.3$ for all frequency bands). However, with progression of exercise there is a significant increase in total spectral power only for the Up phase (Wilcoxon matched pairs test comparing the Up phase at the Beginning vs. End of exercise gives $p < 0.02$ for the low and intermediate frequency bands; see also Fig. 12b, bottom panels). Similarly to the leg muscle in old subjects (Fig. 13b), the Down-phase spectral power for the back muscle (b) does not increase from Beginning to End of exercise, indicating reduced back muscle response to accumulated fatigue during the lengthening contractions of the squat movement and the Down-phase ratio or the old group (c) is close to 1 for all frequency bands. Error bars in plots represent the group average standard error. The red arrows indicate statistically significant changes in spectral power from Down to Up squat phase. Results for the left back (not shown) are consistent with the results for the right back muscle shown in the figure

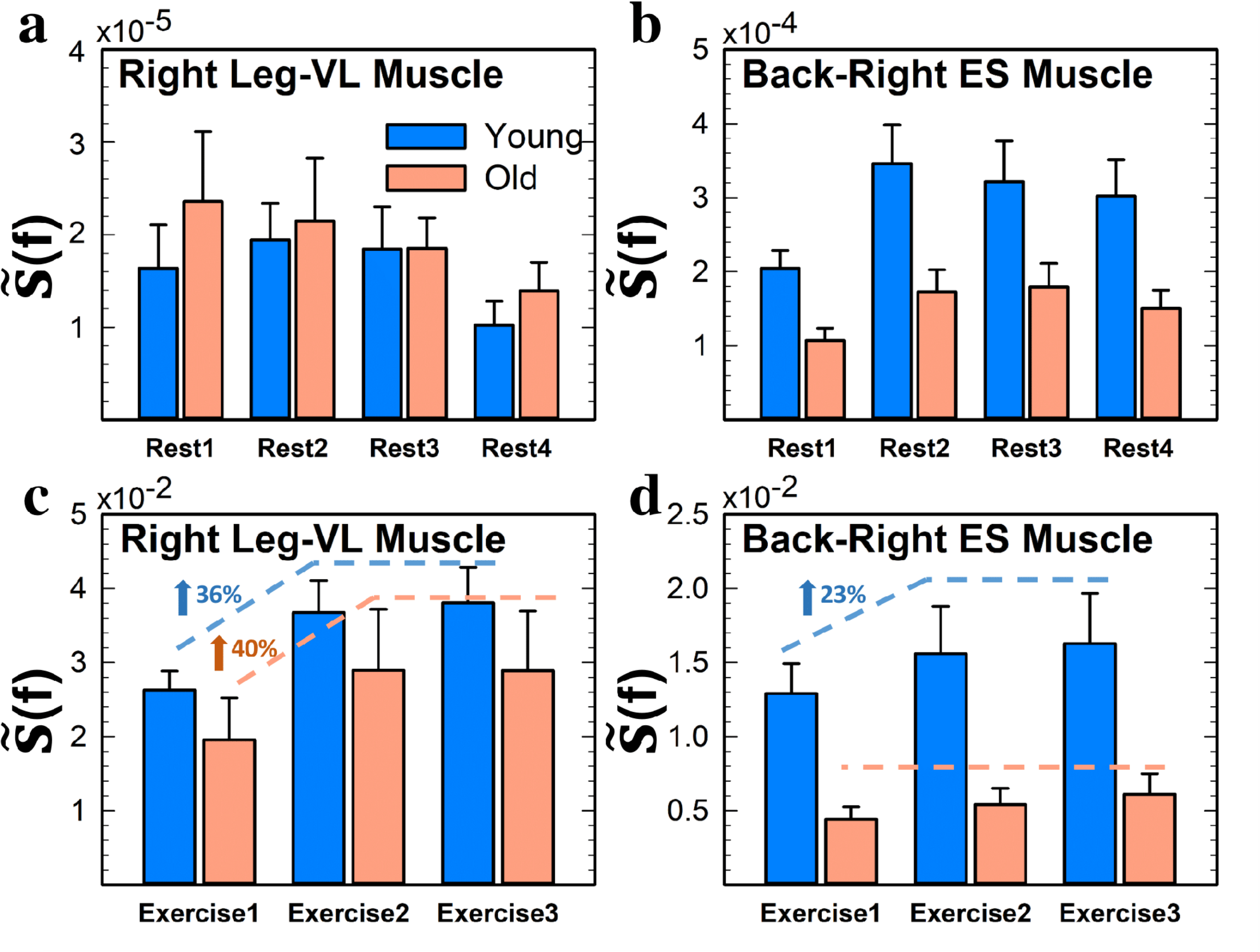


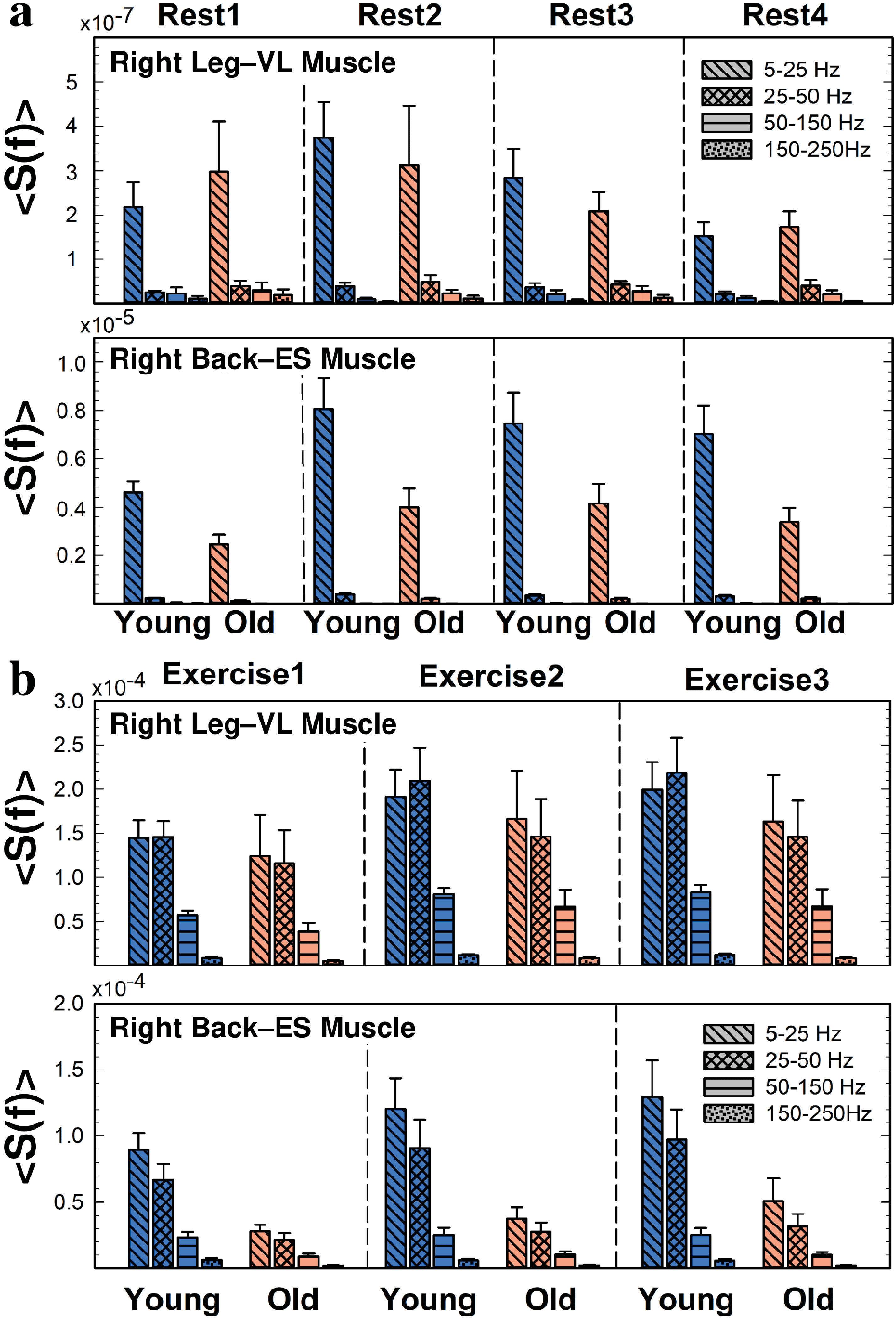
a
Exercise 1
Exercise 2
Exercise 3
Rest 1
Rest 2
Rest 3
Rest 4
VL-L
VL-R
ES-L
ES-R
Left Leg
Right Leg
Left Back
Right Back
0.8
-0.8
0
10
20
30
40
50
b
10.9
11
11.1
11.2
11.3
11.4
Myoelectric Activity, EMG (mV)
Time (min)



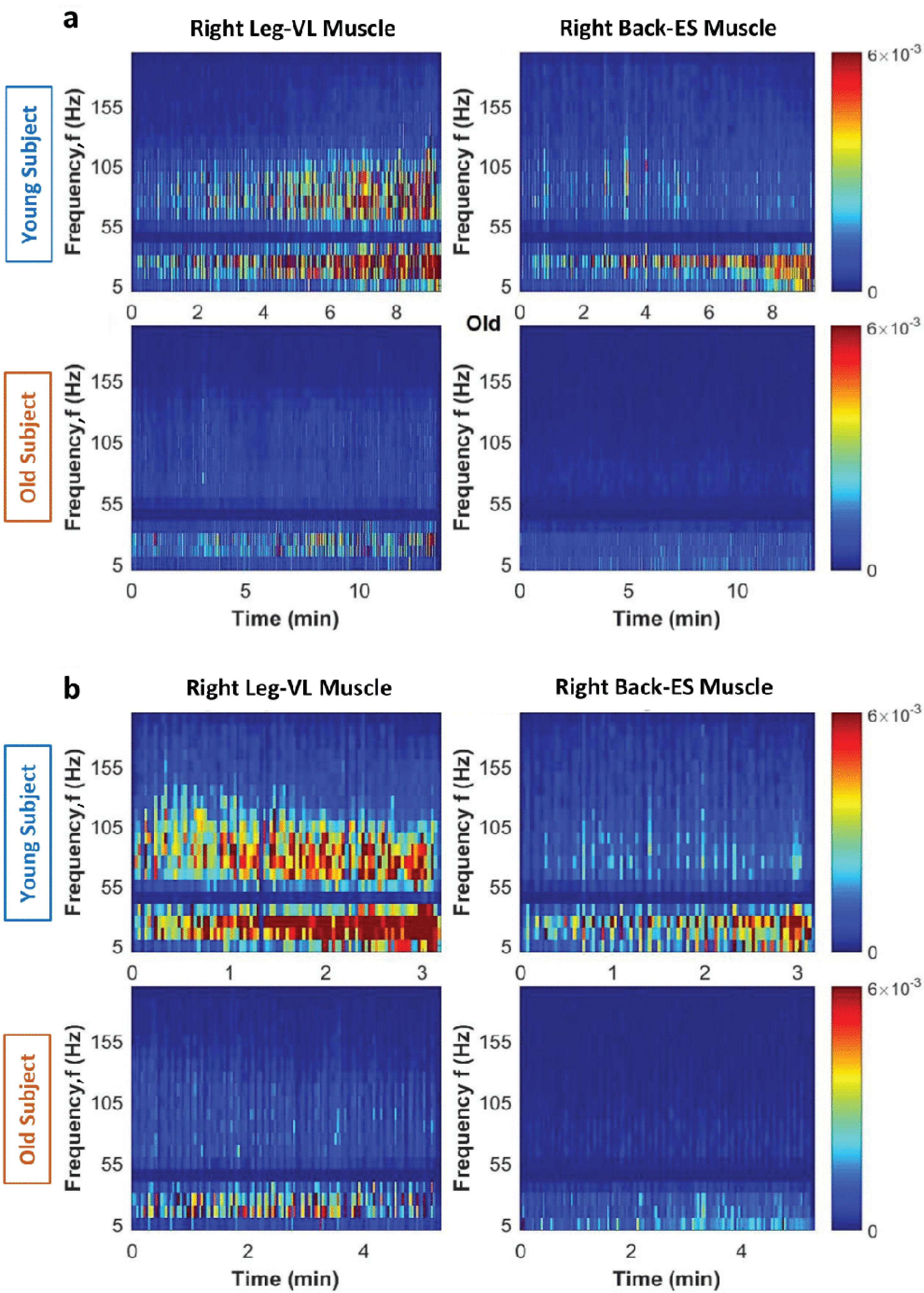
Young Group: Right Leg – VL Muscle
a
×10^-6
1.5
1
0.5
0
S(f)
Rest1
Rest2
Rest3
Rest4
5
50
100
150
200
250
Frequency f (Hz)
Young Group: Right Back – ES Muscle
b
×10^-5
2
1.5
1
0.5
0
S(f)
Rest1
Rest2
Rest3
Rest4
5
50
100
150
200
250
Frequency f (Hz)
Young Group: Right Leg – VL Muscle
c
×10^-4
4
3
2
1
0
S(f)
Exercise1
Exercise2
Exercise3
5
50
100
150
200
250
Frequency f (Hz)
Young Group: Right Back – ES Muscle
d
×10^-4
4
3
2
1
0
S(f)
Exercise1
Exercise2
Exercise3
5
50
100
150
200
250
Frequency f (Hz)

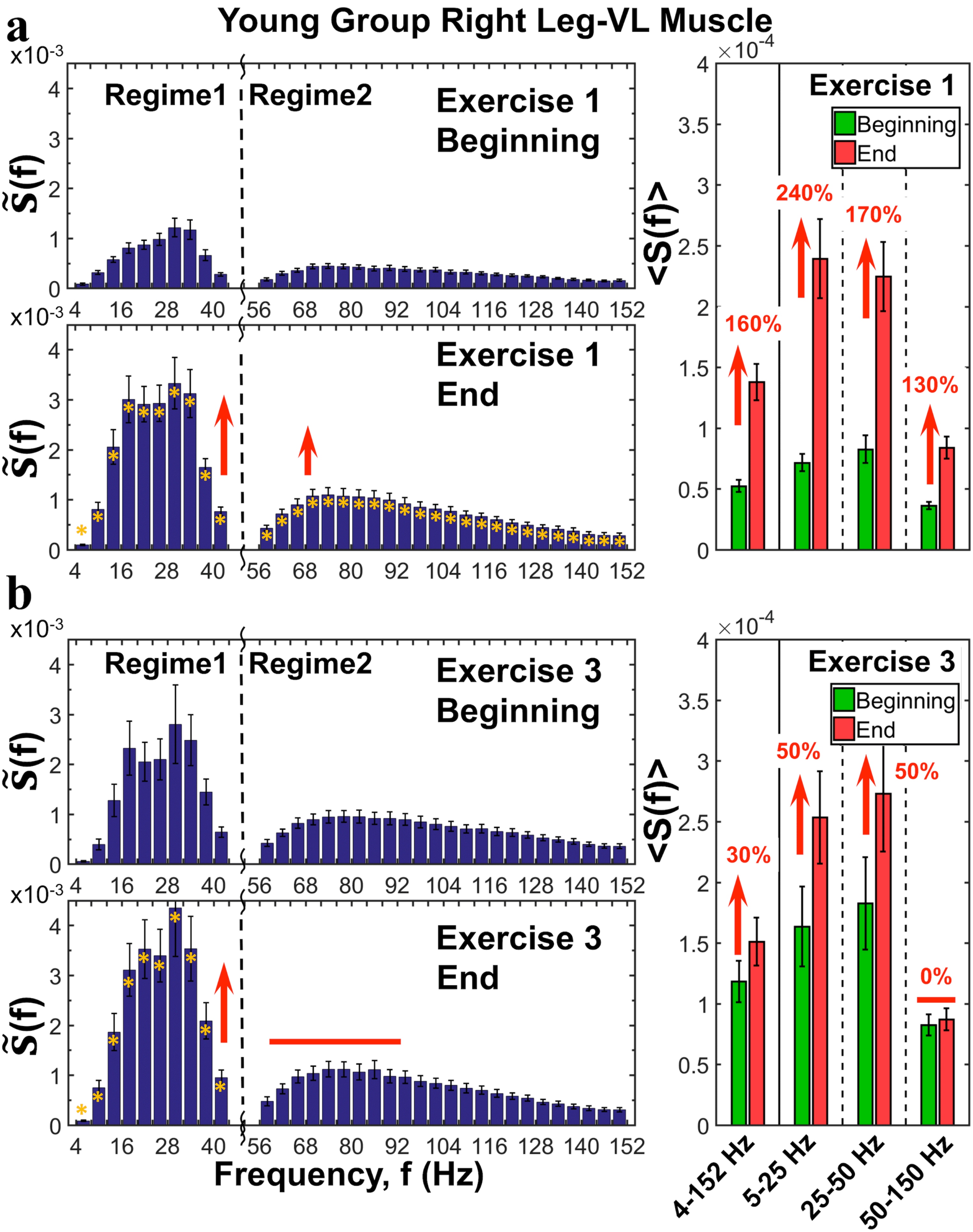

a
Old Group: Right Leg – VL Muscle
×10^-6
Rest1
Rest2
Rest3
Rest4
S(f)
Frequency f (Hz)
b
Old Group: Right Back – ES Muscle
×10^-5
Rest1
Rest2
Rest3
Rest4
S(f)
Frequency f (Hz)
c
Old Group: Right Leg – VL Muscle
×10^-4
Exercise1
Exercise2
Exercise3
S(f)
Frequency f (Hz)
d
Old Group: Right Back – ES Muscle
×10^-4
Exercise1
Exercise2
Exercise3
S(f)
Frequency f (Hz)


a
b
c
d
Right Leg-VL Muscle
Back-Right ES Muscle
Young
Old
$\tilde{S}(f)$
x10-5
x10-4
x10-2
Rest1
Rest2
Rest3
Rest4
Exercise1
Exercise2
Exercise3
36%
40%
23%

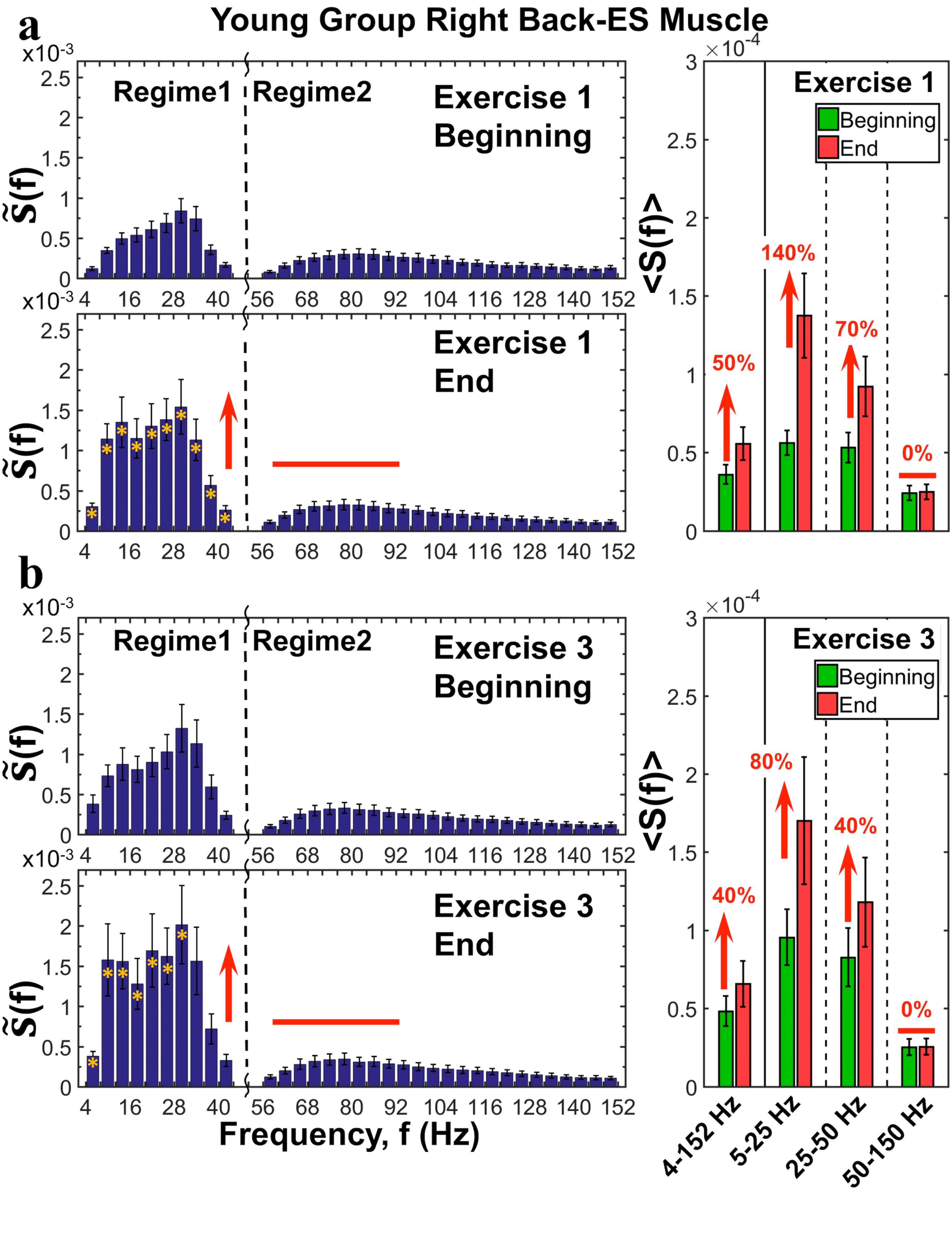


a
Rest1
Rest2
Rest3
Rest4
x10^-7
Right Leg–VL Muscle
5-25 Hz
25-50 Hz
50-150 Hz
150-250Hz
<S(f)>
x10^-5
Right Back–ES Muscle
Young Old Young Old Young Old Young Old
b
Exercise1
Exercise2
Exercise3
x10^-4
Right Leg–VL Muscle
x10^-4
Right Back–ES Muscle
Young Old Young Old Young Old

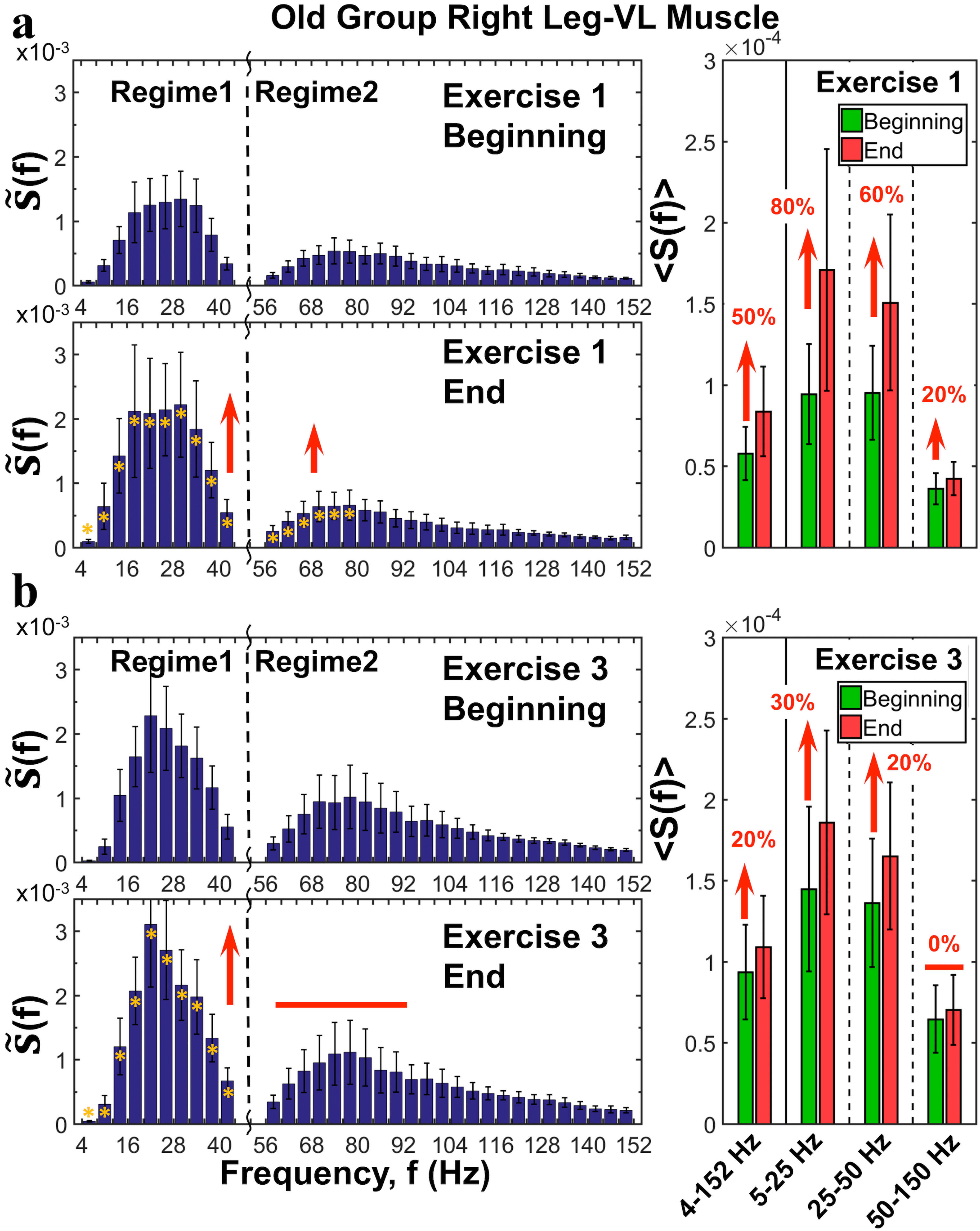

a
Right Leg-VL Muscle
Right Back-ES Muscle
Young Subject
Old Subject
Old
Frequency,f (Hz)
Frequency f (Hz)
155
105
55
5
0
2
4
6
8
10
1
3
6×10^{-3}
Time (min)
b
Right Leg-VL Muscle
Right Back-ES Muscle
Young Subject
Old Subject



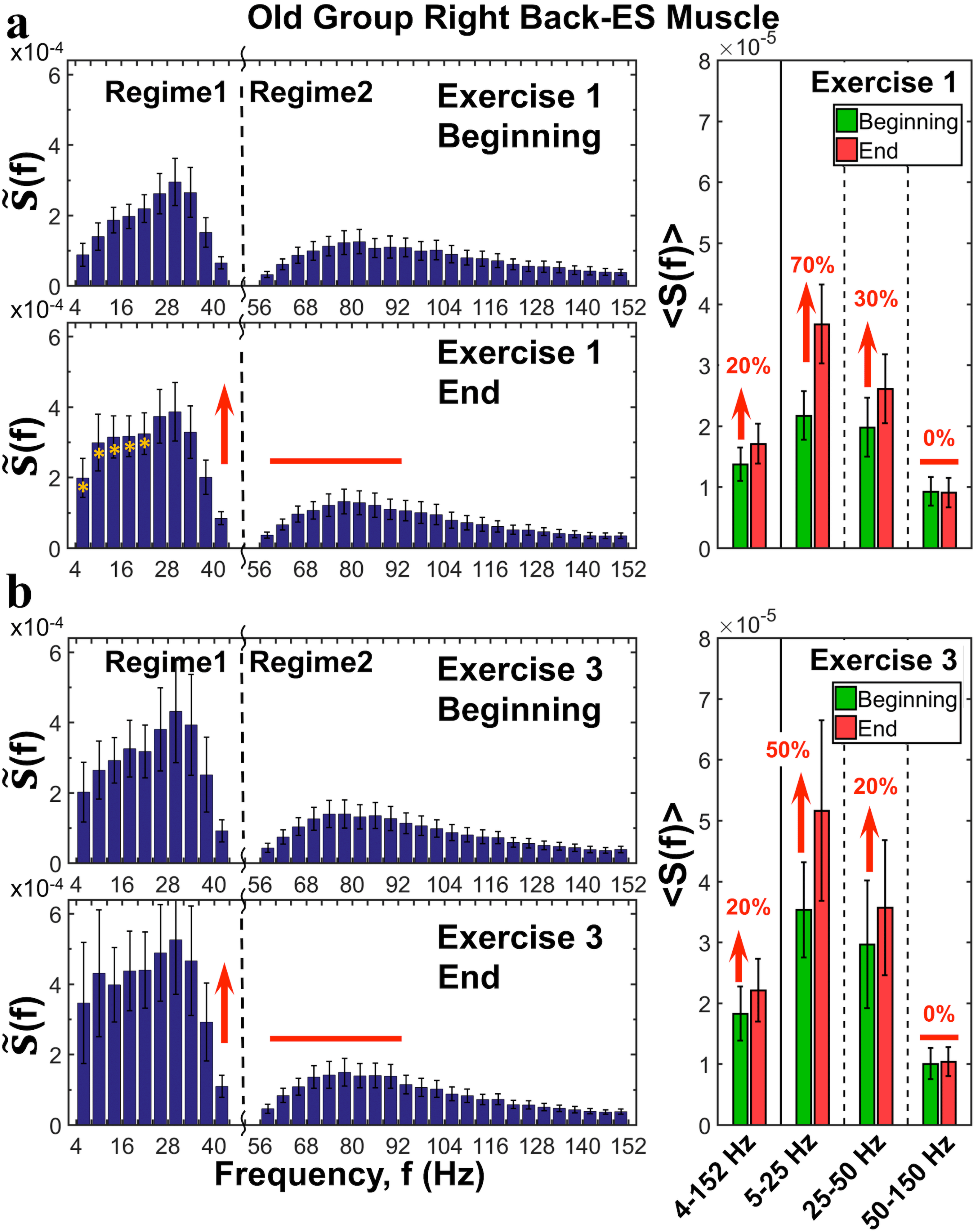
a
Young Group Right Leg-VL Muscle
Regime1
Regime2
Exercise 1
Beginning
Exercise 1
End
x10-3
$\tilde{S}(f)$
$\times 10^{-4}$
<S(f)>
Beginning
End
160%
240%
170%
130%
b
Exercise 3
Beginning
Exercise 3
End
30%
50%
50%
0%
Frequency, f (Hz)
4-152 Hz
5-25 Hz
25-50 Hz
50-150 Hz



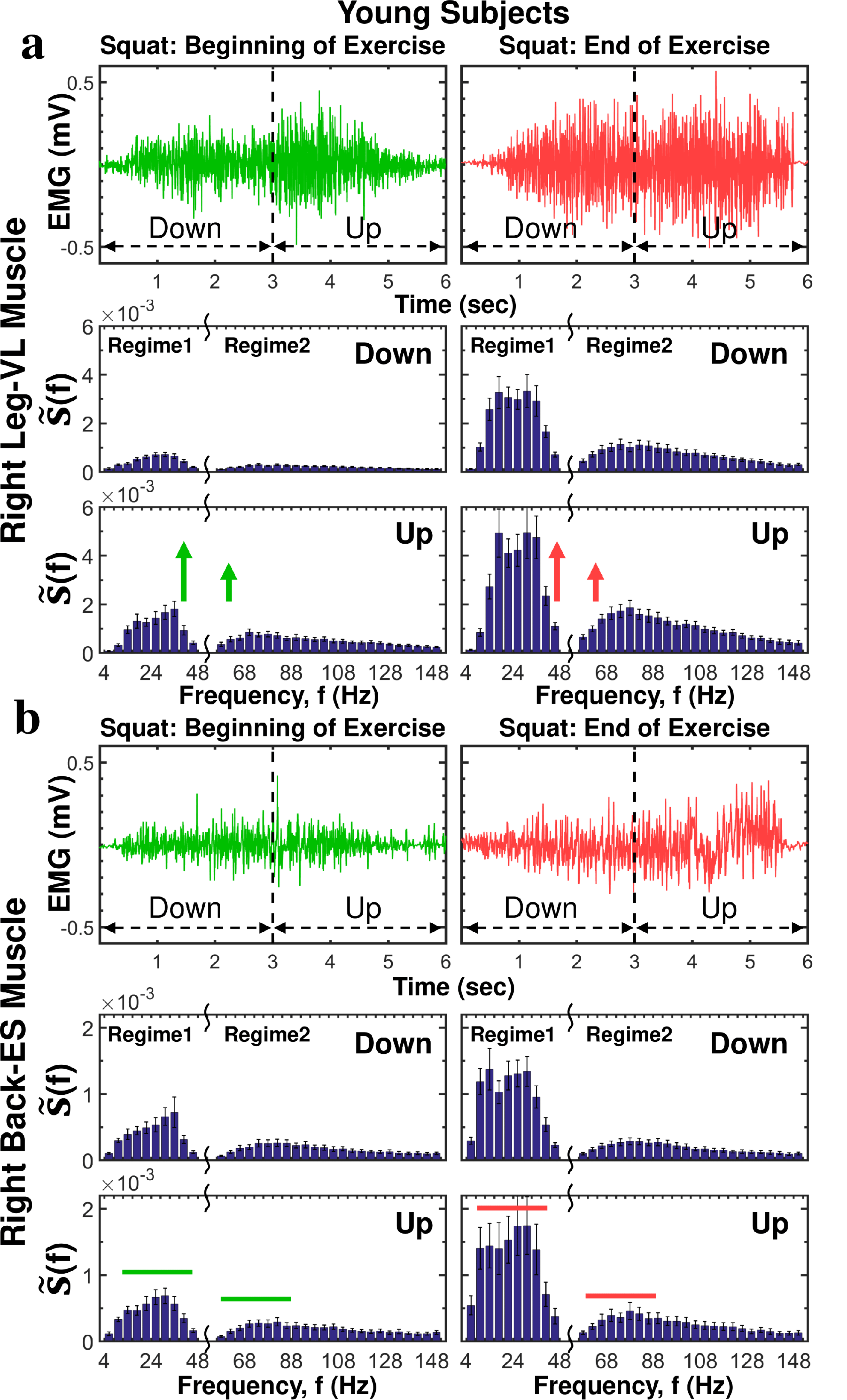
a
Young Group Right Back-ES Muscle
Regime1
Regime2
Exercise 1
Beginning
Exercise 1
End
Exercise 1
Beginning
End
50%
140%
70%
0%
b
Regime1
Regime2
Exercise 3
Beginning
Exercise 3
End
Exercise 3
Beginning
End
40%
80%
40%
0%
Frequency, f (Hz)
4-152 Hz
5-25 Hz
25-50 Hz
50-150 Hz



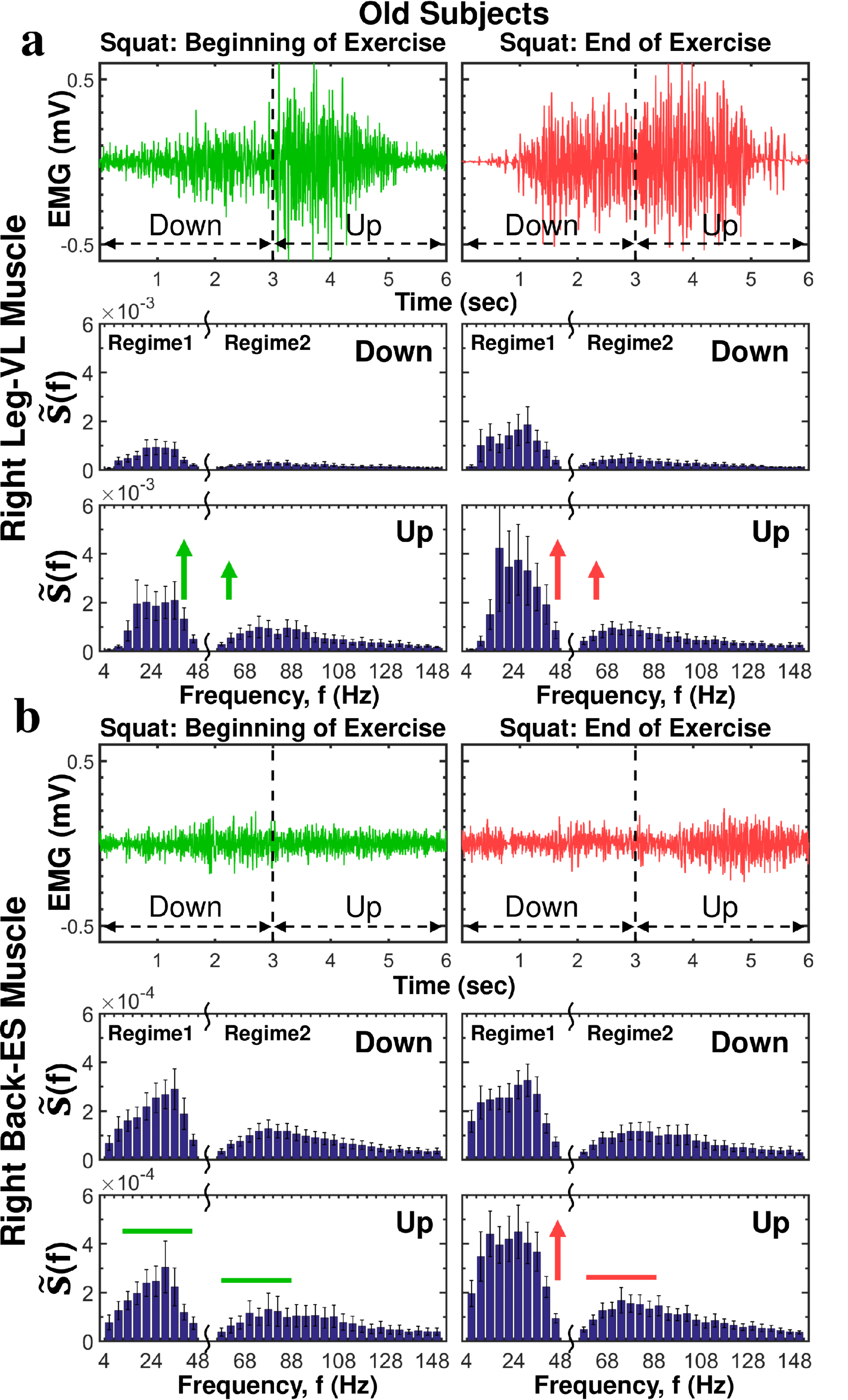
a
Old Group Right Leg-VL Muscle
Regime1
Regime2
Exercise 1 Beginning
Exercise 1 End
Exercise 1
Beginning
End
50%
80%
60%
20%
b
Exercise 3 Beginning
Exercise 3 End
Exercise 3
20%
30%
20%
0%
S̃(f)
<S(f)>
Frequency, f (Hz)
4-152 Hz
5-25 Hz
25-50 Hz
50-150 Hz



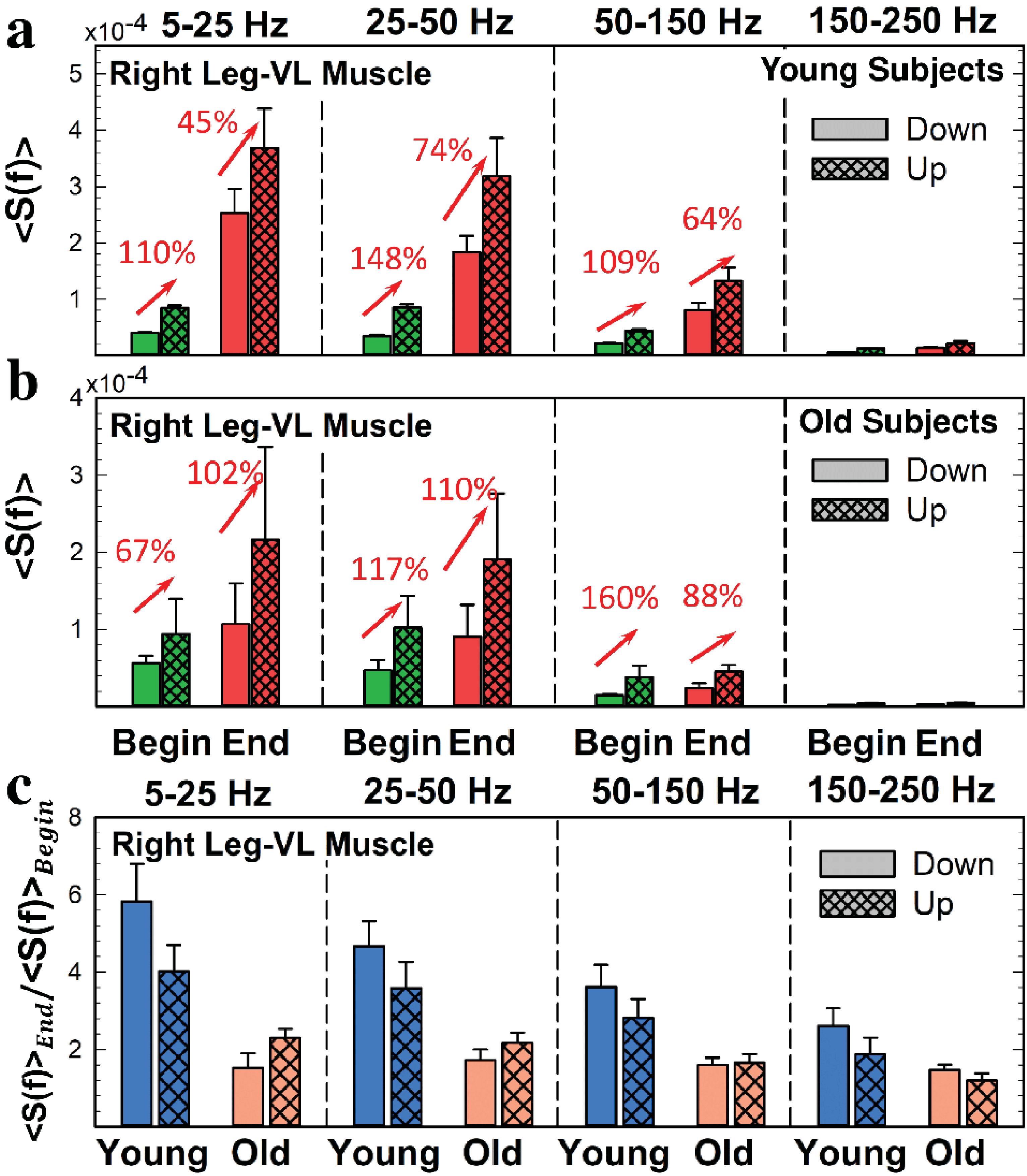
a
Old Group Right Back-ES Muscle
Regime1
Regime2
Exercise 1 Beginning
Exercise 1 End
Exercise 1
Beginning
End
70%
30%
20%
0%
b
Exercise 3 Beginning
Exercise 3 End
Exercise 3
50%
20%
20%
0%
Frequency, f (Hz)
<S(f)>
4-152 Hz
5-25 Hz
25-50 Hz
50-150 Hz

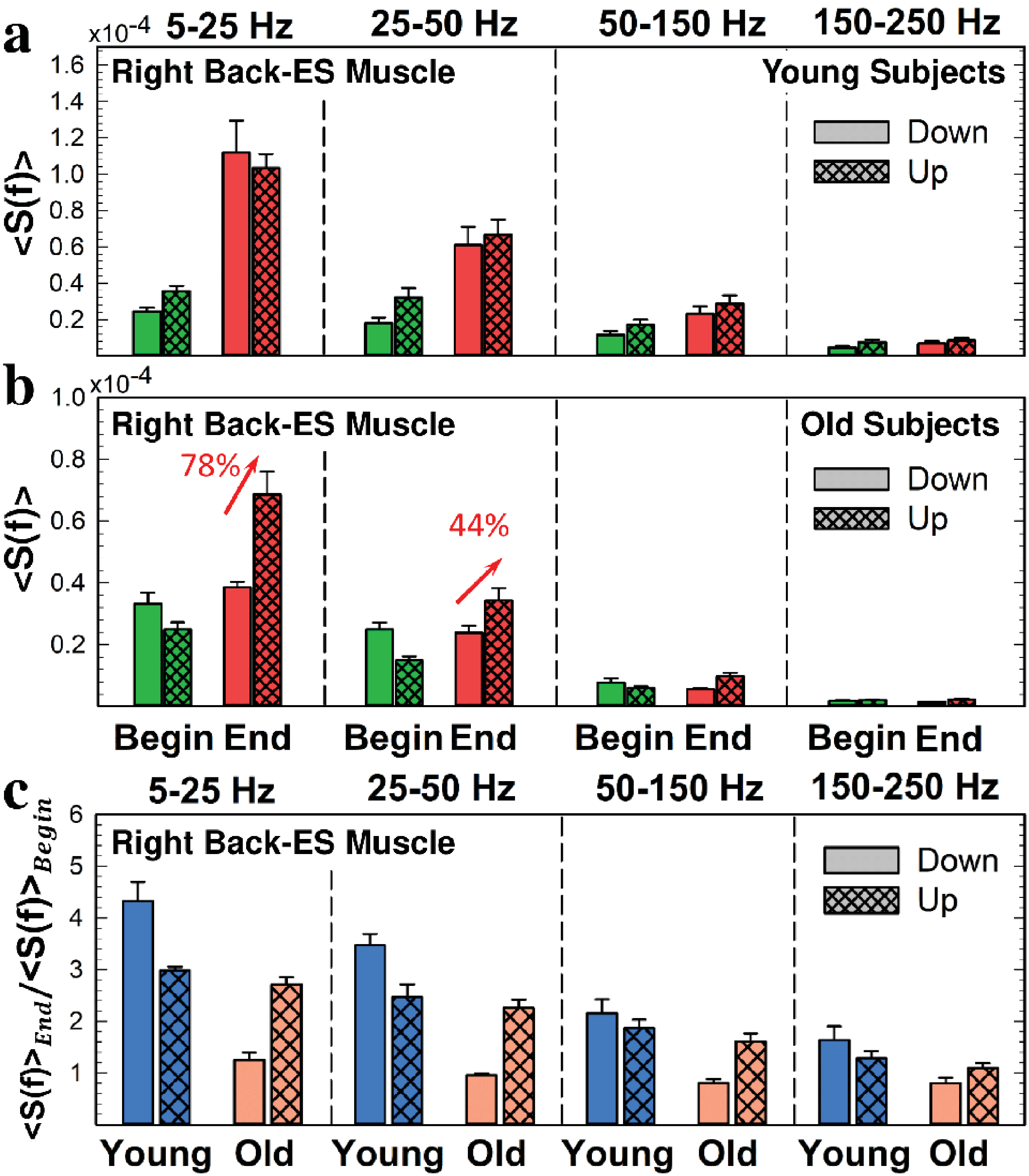


Young Subjects
a
Squat: Beginning of Exercise
Squat: End of Exercise
EMG (mV)
0.5
-0.5
Down
Up
1
2
3
4
5
6
Time (sec)
Right Leg-VL Muscle
×10^-3
6
4
2
0
S̃(f)
Regime1
Regime2
Down
Up
4
24
48
68
88
108
128
148
Frequency, f (Hz)
b
Squat: Beginning of Exercise
Squat: End of Exercise
Right Back-ES Muscle
×10^-3
2
1
0
Regime1
Regime2
Down
Up
Frequency, f (Hz)



Old Subjects
a
Squat: Beginning of Exercise
Squat: End of Exercise
Right Leg-VL Muscle
EMG (mV)
Down
Up
Time (sec)
Regime1
Regime2
Down
Up
S̃(f)
Frequency, f (Hz)
b
Squat: Beginning of Exercise
Squat: End of Exercise
Right Back-ES Muscle
EMG (mV)
Down
Up
Time (sec)
Regime1
Regime2
Down
Up
S̃(f)
Frequency, f (Hz)


a
x10^-4
5-25 Hz
25-50 Hz
50-150 Hz
150-250 Hz
Right Leg-VL Muscle
Young Subjects
Down
Up
110%
45%
148%
74%
109%
64%
<S(f)>
b
x10^-4
Right Leg-VL Muscle
Old Subjects
Down
Up
67%
102%
117%
110%
160%
88%
<S(f)>
Begin End
Begin End
Begin End
Begin End
c
5-25 Hz
25-50 Hz
50-150 Hz
150-250 Hz
Right Leg-VL Muscle
Down
Up
<S(f)>End/<S(f)>Begin
Young Old
Young Old
Young Old
Young Old



a
x10-4
5-25 Hz
25-50 Hz
50-150 Hz
150-250 Hz
Right Back-ES Muscle
Young Subjects
Down
Up
<S(f)>
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
b
x10-4
Right Back-ES Muscle
Old Subjects
Down
Up
<S(f)>
1.0
0.8
0.6
0.4
0.2
78%
44%
Begin End
Begin End
Begin End
Begin End
c
5-25 Hz
25-50 Hz
50-150 Hz
150-250 Hz
Right Back-ES Muscle
Down
Up
<S(f)>End/<S(f)>Begin
6
5
4
3
2
1
Young Old
Young Old
Young Old
Young Old