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Original Article

Preliminary Evidence of Differential Expression of Myogenic and Stress Factors in Skeletal Muscle of Older Adults With Low Muscle Strength

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Abstract

The age-related loss of muscle strength and mass, or sarcopenia, is a growing concern in the aging population. Yet, it is not fully understood which molecular mechanisms underlie sarcopenia. Therefore, the present study compared the protein expression profile, such as catabolic, oxidative, stress-related, and myogenic pathways, between older adults with preserved (8 ♀ and 5 ♂; 71.5 ± 2.6 years) and low muscle strength (6 ♀ and 5 ♂; 78.0 ± 5.0 years). Low muscle strength was defined as chair stand test time more than 15 seconds and/or handgrip strength less than 16 kg (women) or less than 27 kg (men) according to the EWGSOP2 criteria. Catabolic signaling (ie, FOXO1/3a, MuRF1, MAFbx, LC3b, Atg5, p62) was not differentially expressed between both groups, whereas the mitochondrial marker COX-IV, but not PGC1α and citrate synthase, was lower in the low muscle strength group. Stress factors CHOP and p-ERK1/2 were higher (~1.5-fold) in older adults with low muscle strength. Surprisingly, the inflammatory marker p-p65NF-κB was ~7-fold higher in older adults with preserved muscle strength. Finally, expression of myogenic factors (ie, Pax7, MyoD, desmin; ~2-fold) was higher in adults with low muscle strength. To conclude, whereas the increased stress factors might reflect the age-related deterioration of tissue homeostasis, for example, due to misfolded proteins (CHOP), upregulation of myogenic markers in the low strength group might be an attempt to compensate for the gradual loss in muscle quantity and quality. These data might provide valuable insights into the processes that underlie sarcopenia.

Keywords: Muscle aging, Muscle molecular signaling, Muscle strength, Sarcopenia

As society ages and life span extends, the issue of age-related loss of muscle mass and strength—also referred to as sarcopenia—affects an increasing number of older adults (1). It affects up to 29% of community-dwelling older adults and up to 33% of nursing home residents (1). Moreover, sarcopenia predisposes to disability, falls, and hospitalizations (2). In 2019, the European Working group on Sarcopenia in Older People (EWGSOP2) revised their diagnostic criteria for sarcopenia, placing muscle strength upfront as the primary determinant (3). Indeed, low muscle strength (LMS) has been shown to be a better predictor of adverse outcomes and functional decline than muscle mass (4). For example, LMS is associated with an elevated risk of all-cause mortality in older adults (>50 years), regardless of muscle mass (5). Loss of muscle strength has an important clinical impact in the daily life of older adults, whereby almost 20% of women and 10% of men ≥65 years cannot lift a 4.5 kg weight or kneel down (6). Therefore, detecting and treating sarcopenia is of utmost importance to optimize the quality of life in these older adults.

Currently, sarcopenia treatment focuses on resistance exercise (7) whether or not combined with supplements, of which proteins (8) and omega-3 polyunsaturated fatty acids (9, 10) are among the most common ones. However, these therapies can still not fully reverse age-related muscle degeneration. Hence, new therapeutic targets are urgently needed for a potential breakthrough in sarcopenia treatment. To accomplish this, a better understanding of the molecular mechanisms that underlie sarcopenia is required, which can be obtained with muscle biopsies. Whereas the muscle biopsy technique is perceived to be invasive, especially in an older (frail) population, it has proven to be safe, feasible, and well tolerated in older adults and allows to elucidate the molecular mechanisms that underlie age-related sarcopenia (11).

With advancing age, biological (eg, low-grade inflammation (12), anabolic resistance (13), and neural and hormonal changes) as well as behavioral adaptations (eg, nutrition and physical activity) contribute to the muscle phenotype and thus sarcopenia. Whereas the precise mechanisms that underlie the onset and progression of sarcopenia are still not fully understood, it is very likely that molecular networks underlying muscle maintenance and remodeling play a crucial role in the development of sarcopenia (14). In this regard, it is very likely that an upregulation of the major catabolic pathways (ie, ubiquitin–proteasome and autophagy system) and stress factors, as well a downregulation of the anabolic sensitivity (ie, mTORC1 upregulation in response to feeding or exercise), oxidative capacity, and muscle myogenicity might contribute to the sarcopenic muscle phenotype (15, 16).

Most studies that aimed to unravel the signaling pathways responsible for age-related sarcopenia compared the muscle expression profile between young and old animals or humans. However, an important limitation of this approach is that no distinction is made between old muscle with normal functioning and old muscle suffering from impaired functionality or strength, as is the case in sarcopenia. Furthermore, the few reports that compared sarcopenic to nonsarcopenic older adults (17–19) made use of the outdated EWGSOP definition (2010), in which muscle mass and not muscle strength was the primary criterion of sarcopenia (20).

Therefore, in the present study, we aimed to compare the protein expression profile relevant in the context of muscle aging, such as catabolic (eg, FOXO1/3a, LC3b), oxidative (eg, PGC1α, COX-IV), stress-related (eg, ERK1/2, p65NF-κB), and myogenic (eg, Pax7, MyoD) markers between older adults with low compared to preserved muscle strength (PMS). We hypothesized that oxidative and myogenic factors would be downregulated, whereas catabolic and stress-related pathways would be upregulated in older adults with LMS compared to older adults with preserved strength.

Method

Participants and Study Design

Participants were pooled from 2 studies. First, muscle biopsy samples of 10 participants of the ongoing Exercise and Nutrition for Healthy AgeiNg study (ENHANce) at baseline were used. ENHANce (Clinicaltrials.gov: NCT03649698) is a multicomponent (exercise, protein, and omega-3 supplementation), triple-blinded intervention randomized control trial in sarcopenic older adults (21). Second, baseline muscle biopsy samples of 14 participants from the recently published study by Dalle et al. (10) were used. Participants were divided in LMS versus PMS, with LMS being defined as a participant having either a low handgrip strength and/or a prolonged chair stand test according to the cutoff criteria of EWGSOP2 (3). Participants of both studies were selected to have a similar gender ratio and body mass index (BMI) in each group. Accordingly, we aimed for similar age but this was not entirely feasible within this limited study sample. Ethical approval for both ENHANce (S60763) and the Dalle et al. study (S61809) was given by the Ethics Committee Research UZ/KU Leuven. All participants gave written informed consent.

Muscle Strength and Physical Performance Measures

Grip strength was measured with the Jamar 1 hand-held dynamometer (TEC Inc., Clifton, NJ). Maximal grip strength was recorded as the highest of 3 measurements at the dominant side. Participants were considered having low handgrip strength when maximal strength was less than 27 kg for men and less than 16 kg for women, according to the cutoffs proposed by the EWGSOP2 (3). Lower extremity muscle strength was measured with the chair stand test, timing the duration that a participant needs to stand up and sit again for 5 consecutive times (3). Lower extremity muscle strength was considered low when chair stand test time was more than 15 seconds for men and women (3).

To evaluate physical performance, gait speed was determined. This was measured using a 6-m walking test. Participants were instructed to walk 6 m at usual pace (walking aids allowed). The time was recorded over 4 m, that is, starting from the moment the participant's foot passed the mark of the 1-m line until the foot passed the mark of the 5-m line according to the BC guidelines (22). Additionally, the Short Physical Performance Battery (SPPB) was performed to assess function. SPPB is a multicomponent test combining a chair stand test, gait speed, and a balance evaluation. Each domain is scored from 0 to 4 and the total score is 0–12 points (23).

Muscle Biopsy Procedure

Biopsies were performed using the modified Bergström technique (24). In short, a needle biopsy of *musculus Vastus Lateralis* was performed with a 6-mm Bergström needle, using local anesthesia (2% xylocaine or 2% lidocaine), in order to obtain a sample of approximately 150 mg. The samples were taken in the nondominant leg after a 30-minute period of rest. Participants were asked to abstain from exercise 48 hours prior to the muscle biopsy. Blood cloths and adipose tissue were removed from the muscle samples. Muscle samples were immediately frozen in liquid nitrogen to be stored at −80°C.

Protein Extraction and Western Blot

Frozen muscle tissue (10–20 mg) was homogenized 4 × 20 s in ice-cold lysis buffer (1:10 w/v; 50 mM Tris-HCl, pH 7.0; 270 mM sucrose; 5 mM EGTA; 1 mM EDTA; 1 mM sodium orthovanadate; 50 mM glycerophosphate; 5 mM sodium pyrophosphate; 50 mM sodium fluoride; 1 mM dithiothreitol; 0.1% Triton X-100; and a complete protease inhibitor tablet [Roche Applied Science, Vilvoorde, Belgium]) using the FastPrep (MP Biomedicals, Santa Ana, CA). Homogenates were centrifuged (10 000 *g*) for 20 minutes at 4°C and the supernatant was stored at −80°C. Protein concentrations were assessed with the DC protein assay kit applying a BSA protein standard (Bio-Rad Laboratories, Nazareth, Belgium). Lysis buffer was added to equalize protein concentrations, and Laemmli (20% of the total volume) was added to obtain muscle lysates.

Proteins (15–25 µg) were separated by SDS-PAGE (8%–15% gels) and transferred to polyvinylidene difluoride membranes. Thereafter, membranes were blocked in TBS-T (tris-buffered saline with Tween-20) containing 5% nonfat milk or BSA for 1 hour. Next, membranes were incubated overnight at 4°C in TBS-T containing

5% nonfat milk or 5% BSA with the following antibodies: *Catabolism*: phospho forkhead box O1/3a (p-FOXO1/3a; CST-9464S), total FOXO3a (CST-2497S), muscle-specific RING finger protein 1 (MuRF1; sc-32920), muscle atrophy F-box (MAFbx; ECM Biosciences AP2041), microtubule-associated protein 1A/1B-light chain 3 (LC3b; CST-3868), autophagy-related protein 12 (Atg12; CST-4180S), p62 (Progen GP62-C); *Oxidative capacity*: citrate synthase (CST-14309S), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α; sc-517380), cytochrome c oxidase subunit 4 (COX-IV; sc-37673); *Stress*: phospho nuclear factor kappa B (p-p65NF-κB; sc-136548, 1/500), p65NF-κB (sc-372, 1/1 000), C/EBP homologous protein (CHOP; CST-2895S), phospho extracellular signal-regulated kinase 1/2 (ERK1/2; CST-4370), ERK1/2 (CST-4695); *Myogenic capacity*: paired box protein 7 (Pax7; DSHB), MyoD (sc-760), desmin (NBP1-97811). Membranes were then incubated for 45 minutes at room temperature with the appropriate horseradish peroxidase-conjugated secondary antibodies (1/7 000; Sigma Aldrich, Bornem, Belgium). Membranes were scanned and quantified with Genesnap and Genetools Softwares (Syngene, Cambridge, UK). Results are presented as the protein of interest, relative to housekeeping proteins GAPDH (CST-2118S) or vinculin (Sigma, V9131), and/or as the ratio phosphorylated/total form.

Statistics

Descriptive statistics were used to summarize participant baseline characteristics. Comparison of both groups was done with an unpaired *t*-test or a Mann–Whitney *U* test, depending on the distribution of the examined variable (Shapiro–Wilks test). Where relevant, Cohen's *D* was used as an index of effect size (0.2 = small, 0.5 = medium, 0.8 = large). Spearman correlations were applied to assess associations between markers. Significance level was set at $p < .05$, trends were considered when significance was in the range of $p = .05$–.10. Statistics were performed using SPSS version 20 (IBM, Armonk, NY).

Results

Study Participants

Study participants between 65 and 86 years old (mean 74.47 ± 5.06 years) were allocated to the LMS group or the PMS group, based on their chair stand performance and/or grip strength. Participants in the LMS group were significantly older (+8.8%; Cohen's $D = 1.6$; $p = .002$), performed worse on the chair stand test (+63%; $D = 2.3$; $p < .001$), and had a lower gait speed (–30%; $D = 2.2$; $p < .001$) and SPPB score (–29%; $D = 1.5$; $p = .002$), whereas BMI and hand grip strength were not significantly different (Table 1).

Catabolic Signaling

Markers of the 2 most important catabolic pathways were assessed, that is, autophagy (p62, LC3b, Atg12) and the ubiquitin–proteasome system (FOXO1/3a, MuRF1, MAFbx). Contrarily to our hypothesis, none of the catabolic markers were differentially expressed between the PMS and the LMS groups (Figure 1).

Oxidative Signaling

Whereas the major oxidative transcription factor—PGC1α—and the mitochondrial enzyme—citrate synthase—were not differentially expressed in skeletal muscle of the PMS and the LMS groups, the COX-IV enzyme of the mitochondrial electron transport chain tended to be lower in the LMS group compared to the PMS group (–43%; $D = 0.79$; $p = .090$; Figure 2). It should be noted that for PGC1α, there was one significant outlier in the LMS group. After its removal, PGC1α was lower in the LMS group versus the PMS group, but this did still not reach significance ($D = 0.62$; $p = .283$).

Stress Signaling

Muscle stress was assessed on different levels. Regarding inflammation, the phospho/total ratio of the major inflammatory transcription factor p65-NFκB was lower expressed in the muscle of the LMS group compared to the PMS group (~6.5-fold; $D = 0.98$; $p = .019$; Figure 3). In contrast, CHOP, a marker of endoplasmic reticulum stress, was ~1.8-fold higher in the muscle of the LMS group compared to the PMS group ($D = 1.1$; $p = .018$; Figure 3). Accordingly, phospho/total ERK1/2 tended to be increased in the muscle of older adults with LMS (~1.7-fold; $D = 0.54$; $p = .056$; Figure 3). In the PMS group, there was one significant outlier for phospho/total ERK1/2. After its removal, phospho/total ERK1/2 expression was significantly higher in the LMS group versus the PMS group (~2.8-fold; $D = 1.36$; $p = .016$).

Table 1. Baseline Characteristics of the Study Participants

	All Participants	Low Muscle Strength	Preserved Muscle Strength	*p*
Number of participants	24	11	13	
Female (%)	14 (58.3%)	6 (54.5%)	8 (61.5%)	
Age (years ± *SD*)	74.47 ± 5.06	78.01 ± 5.03	71.46 ± 2.58	.002
BMI (kg/m² ± *SD*)	26.9 ± 4.4	27.2 ± 6.0	26.7 ± 2.6	.796
Chair stand test (s ± *SD*)	12.94 ± 5.45	17.49 ± 4.85	9.08 ± 1.49	.001
Grip strength				
All participants	28.3 ± 10.3	26.7 ± 11.2	29.8 ± 9.6	.473
Females	23.0 ± 5.2	22.4 ± 7.1	23.3 ± 3.6	.770
Males	35.9 ± 11.1	31.7 ± 13.8	40.0 ± 6.4	.268
Gait speed (m/s ± *SD*)	1.22 ± 0.24	1.02 ± 0.19	1.38 ± 0.13	<.001
Median SPPB score [range]	12 [5–12]	9 [5–12]	12 [10–12]	.004

Notes: BMI = body mass index; SPPB = Short Physical Performance Battery. Data are presented as mean ± *SD*. Baseline differences between groups were analyzed with either independent samples *t*-tests (age, BMI, grip strength, gait speed) or Mann–Whitney *U* tests (Chair stand test, SPPB).

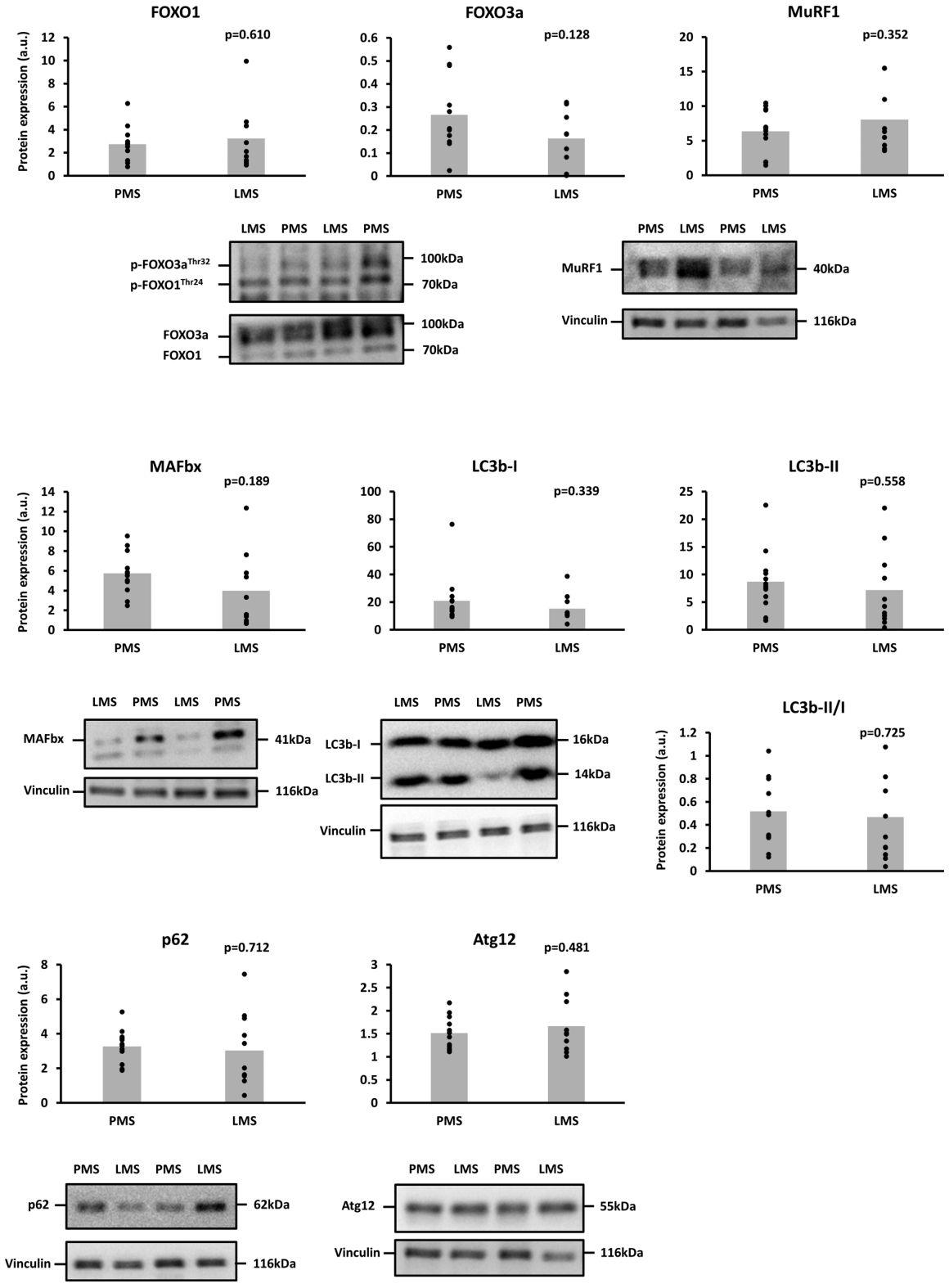

Figure 1. Expression of catabolic markers in skeletal muscle tissue (m. vastus lateralis) of older adults with preserved muscle strength (PMS) and low muscle strength (LMS). Bar graphs represent the average of each condition, and superimposed black dots represent individual data points. Groups were compared with an unpaired *t*-test (normal data). Representative immunoblot protein images of each target are shown for 2 participants per group. FOXO = Forkhead box protein O; MuRF1 = muscle-specific RING finger protein 1; MAFbx = muscle atrophy F-box; LC3b = microtubule-associated protein 1A/1B-light chain 3; Atg12 = autophagy-related protein 12.

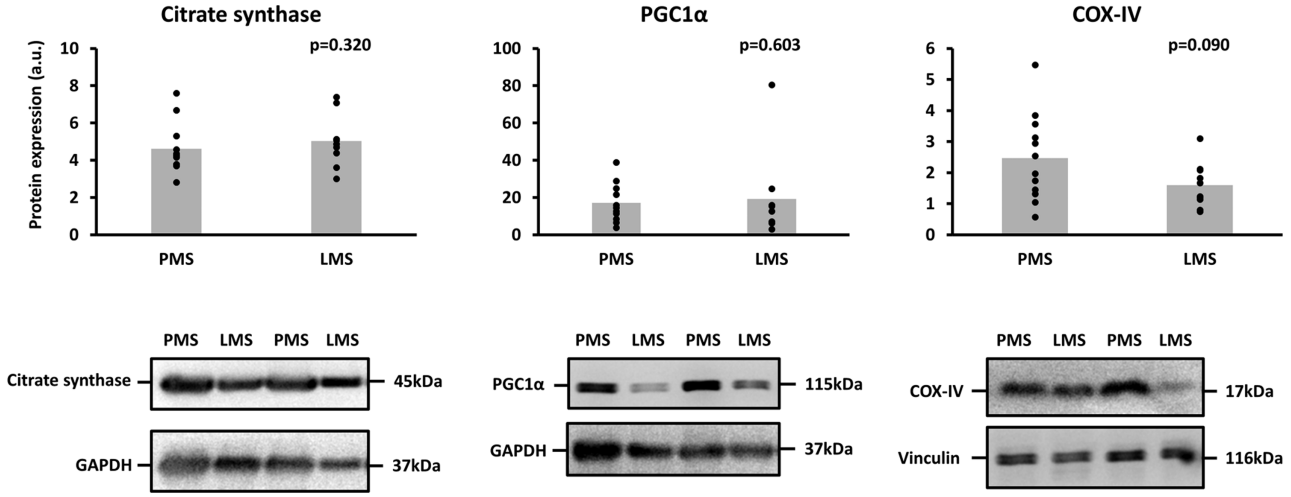

Figure 2. Expression of oxidative markers in skeletal muscle tissue (m. vastus lateralis) of older adults with preserved muscle strength (PMS) and low muscle strength (LMS). Bar graphs represent the average of each condition, and superimposed black dots represent individual data points. Groups were compared with an unpaired *t*-test (normal data: COX-IV) or Mann–Whitney *U* test (non-normal data: PGC1α, citrate synthase). Representative immunoblot protein images of each target are shown for 2 participants per group. PGC1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; COX-IV = cytochrome c oxidase subunit 4.

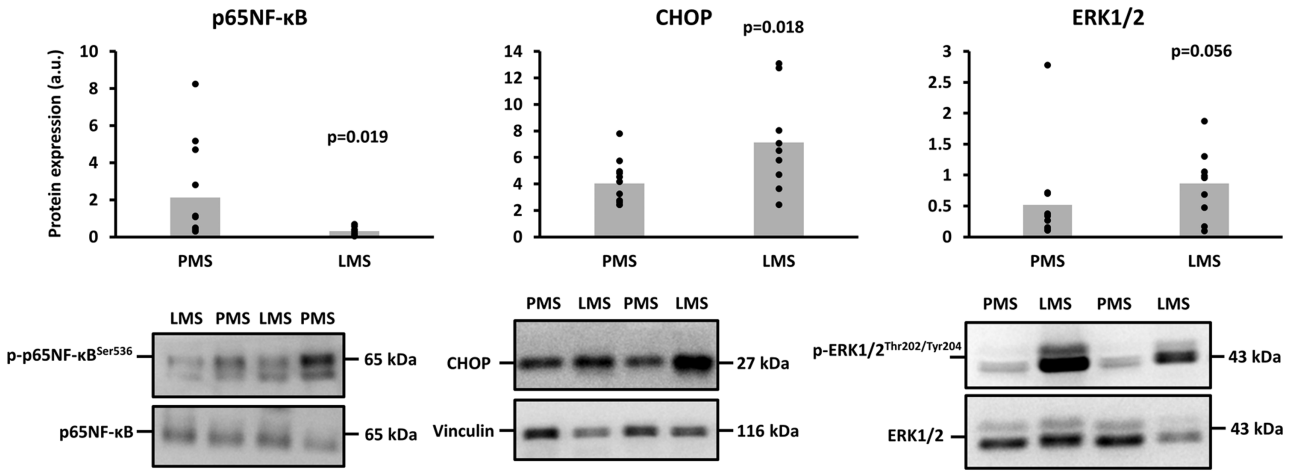

Figure 3. Expression of stress markers in skeletal muscle tissue (m. vastus lateralis) of older adults with preserved muscle strength (PMS) and low muscle strength (LMS). Bar graphs represent the average of each condition, and superimposed black dots represent individual data points. Groups were compared with an unpaired *t*-test (normal data: CHOP) or Mann–Whitney *U* test (non-normal data: p65NF-κB, ERK1/2). Representative immunoblot protein images of each target are shown for 2 participants per group. p65NF-Kb = p65 nuclear factor kappa-light-chain-enhancer of activated B cells; CHOP = C/EBP homologous protein; ERK1/2 = extracellular signal-regulated kinases 1 and 2.

Myogenic Signaling

Markers of muscle myogenicity were significantly higher in the LMS group compared to the PMS group, that is, Pax7 (~2-fold; $D = 0.85$; $p = .030$), MyoD (~2-fold; $D = 0.89$; $p = .022$), and desmin (~3.5-fold; $D = 0.80$; $p = .040$; Figure 4).

Discussion

The present analyses demonstrate that the muscle expression profile of older adults with LMS exhibits fundamental differences compared to that of adults with preserved strength. Whereas catabolic markers were not differentially expressed between both groups, myogenic markers and some stress markers (ie, p-ERK1/2, CHOP) were higher in the muscle of the LMS group. Furthermore, the inflammatory transcription factor p65NF-κB and the mitochondrial marker COX-IV were lower in the LMS group. These findings provide novel insights into the molecular networks that might underlie the age-related loss in muscle strength, which is the primary criterion of sarcopenia.

Few earlier reports already explored the effect of aging on the muscle expression profile. Generally, mitochondrial/oxidative markers are lower at higher age (25–27), while ubiquitin–proteasome related markers (25, 28), autophagy markers (29, 30), and stress markers (31, 32) (eg, muscle inflammation) are more highly expressed in old versus young muscle. Although the aging muscle loses mass, quality, and/or functionality compared to young muscle, an important limitation of this approach involves the fact that young adults are compared with a heterogeneous older population, without distinction between "successful" and "unsuccessful" muscle aging.

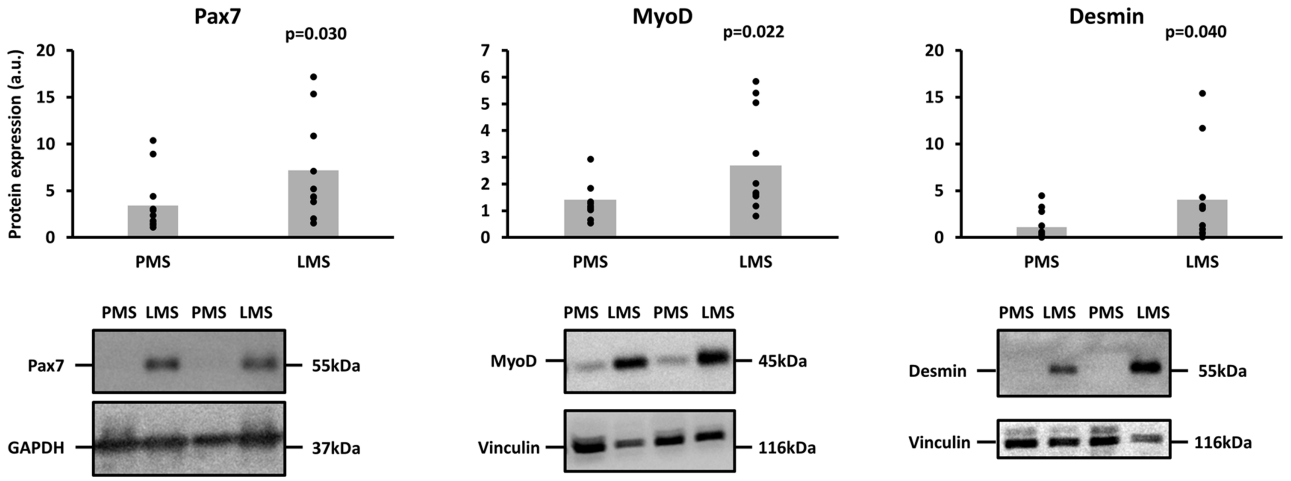

Figure 4. Expression of myogenic markers in skeletal muscle tissue (m. vastus lateralis) of older adults with preserved muscle strength (PMS) and low muscle strength (LMS). Bar graphs represent the average of each condition, and superimposed black dots represent individual data points. Groups were compared with a Mann–Whitney *U* test (non-normal data). Representative immunoblot protein images of each target are shown for 2 participants per group. Pax7 = paired box protein 7.

Hence, this comparison reflects the (tissue) aging as such, rather than the particular mechanisms contributing to the "unsuccessfully" aged muscle phenotype, that is, sarcopenia. Furthermore, with aging, there are changes in physical (in)activity (33) and diet (34), and muscle injuries accumulate. These potential confounding factors might therefore mask the variance in muscle signaling that can be attributed to the loss of muscle mass and/or strength. Hence, an approach in which older adults with low and high muscle strength (the primary determinant of sarcopenia) are compared better reflects the molecular pathways that intrinsically contribute to the sarcopenic muscle phenotype.

Only a few reports studied differences in the muscle expression profile between sarcopenic and nonsarcopenic older adults (17–19). It should be noted that these studies rely on the former EWGSOP definition (2010), in which muscle mass was the primary criterion of sarcopenia, and/or in most of these studies, gene expression rather than more functional protein expression profiles were compared.

Catabolism

In contrast to our data, Marzetti et al. (18) found the protein expression of the autophagy marker LC3b to be lower in sarcopenic versus healthy older adults. Unfortunately, they did not distinguish between the unconjugated (LC3b-I) and the phosphatidylethanolamine-conjugated (LC3b-II) form, which are both required as an estimate for the autophagic flux. Our data revealed no differences between the PMS and the LMS groups in both forms or in their ratio, neither in other autophagy markers.

Oxidative Capacity

Marzetti et al. (18) also found the mitochondrial protein mitofusin-2 to be lower in sarcopenic older adults, whereas other mitochondrial/oxidative markers, such as mitofusin 1 and PGC1α, were not differentially expressed. Accordingly, genome-wide transcriptional changes of sarcopenic versus age-matched controls revealed a lower oxidative phosphorylation and mitochondrial proteostasis in sarcopenia (19), which is partly in line with our observation that the expression of the mitochondrial marker COX-IV tended to be lower in the LMS versus PMS groups.

Stress

Besides catabolic and oxidative markers, also muscle stress markers, including the central proinflammatory transcription factor p65NF-κB, were analyzed. It is not fully understood why older adults with PMS exhibited a higher p65NF-κB signaling compared to older adults with LMS. Cuthbertson et al. (13) found a 4-fold higher total NF-κB expression in the muscle of old versus young (70 vs 28 years) adults. Unfortunately, they did not look at the expression level of the phosphorylated form, which is a more functional measure due to its ability to translocate to the nucleus. Furthermore, other studies concluded that the muscle expression of inflammatory markers (eg, TNFα, interleukin 6 [receptor]) increased with aging (25) and was higher in older adults with low versus high muscle strength (35). Yet, inflammatory signaling (eg, NF-κB and TNFα) was not consistently different between sarcopenic and nonsarcopenic older adults (19). It is possible that the lower p65NF-κB expression in the LMS group might be related to the higher expression of myogenic factors, as p65NF-κB can act as a negative regulator of muscle cell growth (36–39). Spearman correlations in our study indeed indicated a negative association between p-p65NF-κB and the myogenic factors MyoD ($r = -0.508$; $p = .019$) and desmin ($r = -0.682$; $p = .001$) but not Pax7 ($r = -0.347$; $p = .133$).

In contrast to NF-κB and in line with our hypothesis, CHOP and p-ERK1/2 expression were higher in the LMS versus the PMS groups. CHOP expression in different tissues, such as rat muscle (40), was shown to increase with advancing age (41). It is likely that the higher CHOP expression in muscle of LMS versus PMS older adults reflects the activation of the unfolded protein response and thus suggests the presence of misfolded proteins (42). Besides, CHOP might drive a pro-apoptotic response. Yet, it is still under debate whether apoptosis explains age-related muscle wasting in nonpathological conditions (43). Accordingly, p-ERK1/2 was higher in LMS versus PMS. Besides its role as stress sensor, p-ERK1/2 also regulates cell growth (differentiation and proliferation) (44–47), and its increase might therefore be associated with a higher expression of myogenic factors. Indeed, a Spearman correlation revealed that p-ERK1/2 expression positively correlated with Pax7 ($r = 0.458$; $p = .037$), MyoD ($r = 0.494$; $p = .017$), and desmin ($r = 0.579$; $p = .004$).

Myogenic Capacity

We hypothesized that myogenic factors would be lower in the LMS group, as these markers reflect muscle cell growth, and as previous studies showed that Pax7 gene expression was lower in the muscle of old versus young adults (73 vs 31 years) (16) and that satellite cell number was lower (−28%) in old versus young (73 vs 22 years) type II but not type I muscle fibers (48). Unexpectedly, Pax7, MyoD, and desmin were higher expressed in muscle tissue of the LMS group versus the PMS group. Accordingly, Pax7 and MyoD expression, as well as total satellite cell count, was shown to be higher in nonsarcopenic older adults compared to young adults (49, 50). Also, in cancer cachexia, Pax7 expression was higher in muscle cells from patients compared to healthy controls, and in murine cancer cachexia models, Pax7 expression steadily increased during the cachexia progression and preceded the onset of muscle atrophy (36). These data, together with our own findings, advocate that the increased expression of myogenic markers might be a mechanism to compensate for a possible (age-related) loss of muscle mass and/or functionality. It should be noted that a lower satellite cell number might still yield a higher Pax7 expression, depending on their activity status. Either way, from our data and existing literature, it is clear that myogenicity might be an important determinant of age-related loss of muscle mass or functionality and requires further research, especially in humans.

The strength of the present study was that we compared the molecular determinants of age-related sarcopenia between older adults with or without LMS, and as such zoomed in on the mechanisms that underlie sarcopenia and muscle aging. Moreover, to our knowledge, this is the first study that compared protein expression profiles according to well-defined muscle strength cutoffs, suggested by the EWGSOP2 consensus definition of sarcopenia (3). Furthermore, recruiting frail older adults or older adults with functional impairment is challenging and in this perspective, the current sample size of 24 participants is substantial. Yet, some limitations should be considered when interpreting the data of the present study. First, despite that older adults were included in both conditions, the LMS group was still older than the PMS group (~6.5 years; p = .002). Whereas this age difference is far less pronounced than when middle-aged and old-aged adults are compared (~30–40 years), this does not exclude that age might have influenced some outcomes. Second, it cannot be excluded that (lifelong) behavioral differences, such as dietary intake and physical (in)activity, contributed to the differential muscle signaling phenotype. Third, whole-muscle homogenates were used to assess molecular signaling pathways. Therefore, the expression patterns not only reflect signaling of muscle fibers, but also of other mononuclear cell types such as immune cells, endothelial cells, and fibroadipogenic precursor cells. It is increasingly recognized that these cell types, as well as their interaction with muscle cells, are important determinants of the muscle phenotype, which is why future studies should further address signaling events in a cell-specific way. Fourth, although female participants were postmenopausal, this does not exclude that sex differences in the sarcopenia progression during lifetime (eg, due to hormonal regulation) affect signaling at higher age. However, in this study, both women and men were pooled in order to increase the statistical power. Fifth, in the LMS group, most participants were included due to decreased lower extremity strength (chair stand test >15 s), whereas handgrip strength was not significantly different between the LMS and PMS groups. This is compatible with sarcopenia affecting lower limbs earlier than upper limbs (3), but it might have altered the results if muscle strength would be low in both upper and lower extremities of LMS participants.

To conclude, the present study shows that the muscle expression profile of older adults (65–86 years) with LMS is characterized by a higher stress-related (ie, CHOP and p-ERK1/2) and myogenic (ie, Pax7, MyoD, and desmin) signaling, whereas inflammation (ie, p-p65NF-κB) was lower and muscle catabolism was not different from older adults with PMS. These data provide novel indications on the molecular networks behind the progression of age-related sarcopenia. An upregulation of myogenic factors, which might be related to increased p-ERK1/2 and/or decreased p-p65NF-κB expression, seems to be involved in age-related loss of muscle strength. Yet, longitudinal evidence is required (a) to determine whether changes in myogenic factors precede or respond to muscle strength loss, (b) to provide a more detailed view on the changes during the progression of the sarcopenic muscle phenotype, and (c) to eventually investigate whether modulation of myogenic expression affects the clinical muscle phenotype (eg, strength).

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Conflict of Interest

None declared.

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Author Contributions

S.D. and J.D. drafted the manuscript and performed the analyses. L.D., S.V., J.T., E.G., and K.K. provided critical comments and input. All authors read and accepted the final version of the manuscript.

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