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ORIGINAL ARTICLE

Age-specific response of skeletal muscle extracellular matrix to acute resistance exercise: A pilot study

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Abstract
The extracellular matrix (ECM) plays an essential role in the development, growth and repair of skeletal muscles and serves to transmit contractile force. However, its regulation is poorly understood. This study investigates the age-specificity of the effects of acute resistance exercise on ECM gene expression. To this purpose, five young (YM, 23.8 ± 2.2 yrs.) and 5 elderly (EM, 66.8 ± 4.1 yrs.) men performed one session of unilateral leg press and leg extension exercises. Six hours post-exercise, biopsies were taken from the vastus lateralis muscles of both legs. A PCR array was used to profile the expression of 84 ECM-related genes, of which 6 were validated by qPCR. The PCR array found 9 and 4 ECM-associated genes to be selectively altered (>1.5-fold change) in YM or EM only. Four further genes were upregulated in YM but downregulated in EM. Of the 6 genes validated on individual samples MMP9 expression increased in YM (9.7-fold) and decreased (0.2-fold) in EM. MMP15 was downregulated in EM only (0.6-fold). A significant correlation between leg extension 1 RM and changes in COL7A1 expression ($\rho = 0.71$) suggests a potential influence of fitness levels. In conclusion, acute resistance exercise affects ECM gene expression at least partly in an age-specific manner. The altered expression of genes encoding matrix metalloproteinases (MMP3, MMP9, MMP15) highlights the role of remodelling processes in the response to an acute bout of resistance exercise. Larger studies are required to verify the age-associated differences in gene expression profiles and establish their functional implications.

Keywords: *Ageing, resistance training, extracellular matrix, intramuscular connective tissue, matrix metalloproteinases, collagens*

Introduction

Skeletal muscles are characterised by a remarkable capacity to respond and adapt to different types of exercise. In order to explain the mechanisms underlying these changes, the majority of studies have focused on examining molecular pathways inherent to skeletal muscle cells (Hoppeler, 2016). However, many other cell types are embedded in or recruited to skeletal muscles upon stimulation and it is evident that efficient muscle remodelling requires the interplay between these and skeletal muscle cells. As such, tightly regulated inflammatory processes involving neutrophils, macrophages, mast cells, eosinophils, cytotoxic T cells, and T-regulatory lymphocytes contribute to exercise adaptations (Peake, Neubauer, Della Gatta, & Nosaka, 2017). Slightly less is known about the exact role of fibroblasts which have been shown to directly stimulate the proliferation, differentiation and fusion of myogenic precursor cells (Mackey, Magnan, Chazaud, & Kjaer, 2017). Most importantly, fibroblasts and other adherent cells are sensitive to mechanical strains in their extracellular matrix (ECM) environment and able to transduce mechanical into chemical

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information and finally into specific changes in gene expression (Chiquet, Gelman, Lutz, & Maier, 2009). Not surprisingly, the focus of scientific research has been expanded to the ECM surrounding the muscle fibres in order to understand the complex processes of muscle adaptation, regeneration and repair (Mackey & Kjaer, 2017b).

Classically, the muscular ECM is subdivided into endomysial (around the muscle cell), perimysial (around groups of muscle cells), and epimysial (around the whole muscle) connective tissues building a complex architecture which involves numerous collagens, laminins, proteoglycans, and various other proteins (Gillies & Lieber, 2011). Remodelling of the ECM is an integral process of skeletal muscle stem cell activity to support propagation and self-renewal (Rayagiri et al., 2018). A so-called "transitional matrix" characterised by an upregulation of tenascin-C, hyaluronic acid and fibronectin has been identified during the regeneration of amputated limbs in newts (Calve, Odelberg, & Simon, 2010). In humans, the basement membrane has recently been shown to play an important role in the regeneration of muscle subjected to electrically-induced lengthening contractions (Mackey & Kjaer, 2017a). Simultaneously, ECM components, such as collagen, proteoglycans and glycoproteins, are degraded by matrix metalloproteinases (MMPs), particularly gelatinase A (MMP-2) and gelatinase B (MMP-9), thereby contributing to the remodelling of the ECM (Carmeli, Moas, Reznick, & Coleman, 2004).

At older age, skeletal muscles typically demonstrate fibrotic morphology (Lieber & Ward, 2013), where the clear two-directional lattice orientation of healthy perimysial collagen fibres is lost and replaced by an erratic fibre network featuring decreased crimp formation (Jarvinen, Jozsa, Kannus, Jarvinen, & Jarvinen, 2002). Also, absolute collagen content and (non-enzymatic) cross-linking of collagen fibres (Haus, Carrithers, Trappe, & Trappe, 2007) may be increased, leading to progressive muscle stiffening (Wood et al., 2014). While the mechanisms are not yet fully understood, these changes are believed to impair muscle fibre contractility (Azizi, Deslauriers, Holt, & Eaton, 2017) and hinder lateral force transmission (Sharafi & Blemker, 2011). Progressive resistance training is generally considered the most effective and widely applicable tool to counter age-associated muscle function deficits (Montero-Fernandez & Serra-Rexach, 2013). While training programmes as typically prescribed for older adults (American College of Sports Medicine et al., 2009) have been shown to be effective in inducing substantial strength gains and, albeit to a lesser degree, muscle hypertrophy (Frontera & Bigard, 2002), human studies investigating the potential of physical training to stimulate remodelling of the ECM network are scarce and involved only young participants.

In this respect there is some evidence that various forms of acute exercise such as 1 h of one-legged kicking exercise at 67% of maximum workload (Miller et al., 2005), a bout of 100 maximum voluntary eccentric contractions of the knee extensors (Mackey, Donnelly, Turpeenniemi-Hujanen, & Roper, 2004), and resistance exercise consisting of 10 sets of knee extensions against both light (16% of the one-repetition maximum (1RM)) and heavy loads (70% of 1RM) (Holm et al., 2010) have the potential to stimulate collagen synthesis in skeletal muscle. It has also been suggested that the reaction of the ECM to physical training may depend on contraction mode, with eccentric contractions triggering significantly greater fractional synthesis rates of ECM-related collagens as compared to concentric ones (Holm, Rahbek, Farup, Vendelbo, & Vissing, 2016). Such remodelling of the ECM might help to protect muscles against injury as previous exposure to electrical stimulation or lengthening contractions has been shown to lead to an attenuated response at the second bout (Hyldahl et al., 2015). Yet, it is unclear whether the results obtained in these studies could be extrapolated to senior cohorts.

For these reasons, this pilot study aimed to examine potential differences in the adaptation of the ECM to an acute bout of resistance exercise between age groups. Using a within-subject study design, we determined changes in the expression of ECM-associated genes to test our hypothesis that acute exercise would induce or repress genes responsible for ECM turnover. Considering reports of skeletal muscle fibrosis at older age (Lieber & Ward, 2013), we further expected an age-specific response pattern of genes involved in ECM remodelling.

Materials and methods

Subjects and study design

Five young (age: 22.6 [20.1–26.0] years) and 5 older subjects (age: 68.3 [59.8–69.2] years) volunteered to participate in this study. All subjects were recreationally active but not specifically strength-trained (no history of systematic strength training within 6 months prior to inclusion into the study). Physical readiness for participation was assessed by the PAR-Q questionnaire (Warburton et al., 2011). Subjects suffering from coronary heart disease, pulmonary or systemic arterial hypertension (>160/100 mmHg), coagulation disorders, acute infections and moderate to severe osteoarthritis of the knee were excluded from participation. This study was approved by the Institutional Review Board of the University of

Innsbruck (vote AN2016-0038) and conducted in agreement with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki.

Anthropometric data were collected by standard methods in minimal indoor clothing and without shoes. Body mass was measured to the nearest 0.1 kg (Kern DS 150K1, Kern & Sohn GmbH, Balingen-Frommern, Germany) and height was measured to the nearest 0.5 cm using a portable stadiometer (Seca 217, Seca GmbH & CoKG, Hamburg, Germany). Body mass index (BMI) was calculated as body mass relative to height squared (kg/m^2). All participants were subjected to a single session of resistance exercise targeting the knee extensor muscles of a single leg only. The exercise session was scheduled early in the morning to minimise diurnal variations in gene activity and potential bias due to precedent physical activity. The exercised leg was randomised to be the dominant or non-dominant leg, respectively.

Resistance exercise protocol

Following 5 min of general warm-up on a cycle ergometer and submaximal familiarization trials, subjects were requested to complete 3 × 12 repetitions on commercially available knee extension and leg press training devices (Leg Extension Med and Leg Press Med, TechnoGym, Cesena, Italy). One minute of passive recovery was granted between sets. The exercise intensity was set to 70% of the individual one-repetition maximum (1-RM), which was determined in a separate test performed 48 h prior to the actual exercise session. All testing and exercise sessions were supervised by an experienced investigator.

Sampling and analysis of muscle biopsies

Six hours after cessation of exercise, muscle biopsies were taken from the vastus lateralis muscle of both the trained and non-trained leg. The following procedure was applied for both legs: Fifteen centimetres proximal of the proximal margin of the patella, the biopsy site was identified at the anterolateral thigh. After local anaesthesia of the skin with lidocaine the skin and subcutis were incised over a length of approximately 5 mm. On each leg a 2.1 mm biopsy needle (HistoCore, Bard Biopsy Systems, Tempe, AZ, US) was inserted twice into the same incision site to harvest tissue from the vastus lateralis. Biopsy samples were separated from any fatty tissue and blood, immediately dispersed in Allprotect® Tissue Reagent (QIAGEN GmbH, Hilden, Germany), stored at 2–8°C for seven days and then at −80°C until further analysis. Total RNA was extracted from ~20 mg of muscle tissue using the miRNeasy kit (QIAGEN GmbH, Hilden, Germany) according to instructions provided by the manufacturer. Samples were disrupted directly in 700 µl of Qiazol provided with the kit using the TissueLyser II (QIAGEN GmbH, Hilden, Germany) operating at 25 Hz for 3 intervals of 2 min with a timed break of 2 min between the intervals. Following chloroform extraction total RNA was purified using silica-based spin columns. Total RNA was quantified on a spectrophotometer (NanoDrop ND-1000, PeqLab Biotechnologie GmbH, Erlangen, Germany). Purity was checked by calculating 260/280 ratios which were 1.94 [1.80–1.97]. One µg of RNA was reverse transcribed using the high Capacity RNA-to-cDNA™ kit (Applied Biosystems, Foster City, CA) and stored for further analyses at −40°C.

PCR array for human extracellular matrix and adhesion molecules

In order to profile the expression of 84 genes related to the extracellular matrix, equal amounts of total RNA were pooled to obtain four different samples (young-untrained, young-trained, old-untrained, old-trained) which were subjected to analyses by the TaqMan® Array for Human Extracellular Matrix & Adhesion Molecules (Applied Biosystems, Foster City, CA, Catalog # 4414133). Each well of the Array Plate was reconstituted using a mix of TaqMan® Gene Expression Master Mix (Applied Biosystems, Foster City, CA) and the respective cDNA sample (25 ng/well) to a final volume of 20 µl. RNA expression levels were quantified on a 7500 real time PCR system (Applied Biosystems, Foster City, CA) using standard thermal cycling conditions. Expression levels were normalised to the average of four housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin beta (ACTB), glucuronidase beta (GUSB), ribosomal protein lateral stalk subunit P0 (RPLP0)), selected for the most consistent C_T values among the four conditions. A list of all analysed genes including raw C_T data can be found in supplementary file 1.

Quantitative real-time PCR

Genes which were induced or repressed by exercise (>1.5-fold) were selected from the array data set. Specifically, 2 genes of each of the following observation categories were selected: down- or up-regulated in only one age group, down-regulated in both age groups, up-regulated in young but down-regulated old subjects. As collagens and matrix metallopeptidases were represented multiple times in the final gene list, we arbitrarily decided to focus in this

pilot study on these groups of genes rather than including others such as SPP1 or CTNND2, which featured higher or lower –fold changes. Finally, the selected genes were validated on the individual samples using quantitative real-time PCR. Gene specific primers and probes as provided with the commercially available TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA) were used to assess the expression of the following genes: collagen type I alpha 1 chain (COL1A1, Hs00164004_m1), collagen type VII alpha 1 chain (COL7A1, Hs00164310_m1), ADAM metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1, Hs00199608_m1), matrix metallopeptidase 3 (MMP3, Hs00968305_m1), matrix metallopeptidase 9 (MMP9, Hs00957562_m1), matrix metallopeptidase 15 (MMP15, Hs00233997_m1), ACTB (Hs01060665_g1) and GAPDH (Hs02786624_g1). PCR reactions consisting of the cDNA template (25 ng/well), the gene-specific primers/probe set and the TaqMan® Gene Expression Master Mix were prepared in a total volume of 10 µl according to the manufacturer's instructions. Each sample was measured in triplicates on 384-well plates. Skeletal muscle RNA (AM7982, Life Technologies GmbH, Darmstadt, Germany) served to prepare the respective standard curves (0.14–100 ng per reaction). Standard thermal cycling conditions were applied using the QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA). Data were normalised to the mean of the two housekeeping genes GAPDH and ACTB in order to compensate for variations in efficiency of the reverse transcription.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics Version 23 (IBM Corporation, NY, USA). Differences in general participant characteristics between young and old were determined by Mann–Whitney U tests, whereas differences between trained and untrained legs were calculated by Wilcoxon tests. Effect sizes (r) were calculated by dividing the standardised test statistics (Z) by the square root of the number of observations. Figures and tables show individual data points and the median value [min – max], respectively. Associations between different variables were described by Spearman's rho correlation coefficients. For all analyses, significance was accepted at $p < 0.05$.

Results

Subject characteristics

The subjects' anthropometric characteristics and performance measures are summarised in Table I. Body height, body mass and BMI were not significantly different between young and old subjects. As evident from Table I, relative 1-RM loads, calculated through normalisation to body mass, were greater in young subjects by 52.9% (leg extension, $p = 0.056$) and 35.5% (leg press, $p = 0.016$), respectively. Absolute 1-RM loads were non-significantly higher in young subjects by 8.3% (leg extension, $p = 0.151$) and 23.5% (leg press, $p = 0.151$), respectively.

Extracellular matrix gene expression profiling

The results of the Extracellular Matrix & Adhesion Molecules array demonstrated differences in exercise-induced gene expression changes between young and old subjects. In young males, acute resistance exercise led to a selective up-regulation (≥ 1.5-fold change) of 7 genes, whereas 2 genes were down-regulated (≤ 0.67-fold change). In older subjects only 2 genes were up-regulated and 2 genes were decreased after resistance training. Four further genes displayed differential expression in young (up-regulation) and old (down-regulation) subjects (Table II). The genes identified to be

Table I. Subject characteristics.

	Young ($n = 5$)	**Old** ($n = 5$)	p-value
Age (years)	22.6 [20.1–26.0]	68.3 [59.8–69.2]	*0.008*
Height (m)	1.79 [1.69–1.91]	1.78 [1.77–1.90]	*1.000*
Body mass (kg)	72 [64–83]	78 [72–111]	*0.151*
BMI (kg/m^2)	21.5 [20.4–25.9]	23.4 [23.0–30.7]	*0.056*
1-RM leg extension (kg)	56.3 [54.0–76.5]	51.8 [38.3–59.0]	*0.151*
Relative 1-RM leg extension (–)	0.84 [0.75–1.11]	0.49 [0.47–0.82]	*0.056*
1-RM leg press (kg)	95 [90–110]	75 [50–109]	*0.151*
Relative 1-RM leg press (–)	1.38 [1.25–1.49]	0.96 [0.68–1.32]	*0.016*

Note: Data represent median [minimum – maximum]; p-values as calculated by Mann-Whitney U-tests between young and old group.
Abbreviations: BMI (body mass index); 1-RM (1 repetition maximum).

Table II. PCR array-based screening of ECM gene expression changes in response to resistance exercise.

Gene symbol	Young (-fold change from untrained)	Old (-fold change from untrained)
Down young OR old		
COL7A1	**0.62**	0.85
SELE	**0.67**	0.69
MMP15	0.88	**0.59**
CTNND2	0.88	**0.35**
Up young OR old		
ITGB3	**1.54**	1.10
COL1A1	**1.56**	1.03
ITGAL	**1.57**	0.95
CD44	**1.60**	1.28
LAMB3	**1.63**	0.75
CDH1*	**3.36**	0.73
SELL	**4.74**	0.74
ADAMTS1	1.34	**2.10**
ADAMTS8	1.02	**1.61**
Up young AND down old		
MMP3	**2.03**	**0.37**
LAMA1*	**2.07**	**0.32**
MMP9	**8.05**	**0.27**
SPP1	**12.87**	**0.36**

Note: Data represent –fold changes from untrained in young and old subjects as determined by TaqMan® Array for Human Extracellular Matrix & Adhesion Molecules. Genes that are either up- or down-regulated at least 1.5-fold are marked in bold, whereby a value ≤ 0.67 represents an at least 1.5-fold down-regulation. Asterisks denote genes with C_T values above 35 in all samples.

responsive to resistance exercise in young and/or old individuals included matrix metallopeptidases (MMP3, MMP9, MMP15), ADAM metallopeptidases (ADAMTS1, ADAMTS8), collagens (COL7A1, COL1A1), integrins (ITGB3, ITGAL), laminins (LAMB3, LAMA1), and other cell adhesion molecules (CD44, CDH1, SELE, SELL, CTNND2, SPP1). The individual results of the PCR array are shown in the supplementary file 1 [Raw data PCR array_S1.xlsx].

Resistance-exercise induced changes in ECM gene expression

To validate the data obtained by the PCR array, the gene expression levels of COL7A1, COL1A1, ADAMTS1, MMP3, MMP9, and MMP15 were determined by real-time quantitative RT-PCR on the individual samples. An acute bout of resistance exercise was found to significantly decrease COL7A1 gene expression levels (untrained leg: 1.36 [0.90–2.70], trained leg: 1.24 [0.76–1.84], $Z = -2.50$, $p = 0.013$, $r = -0.79$, corresponding to a 0.83 [0.67–1.01] -fold change), irrespective of age group. In contrast, ADAMTS1 gene expression levels were significantly increased (untrained leg: 0.142 [0.105–0.186], trained leg: 0.167 [0.145–0.386], $Z = 2.50$, $p = 0.013$, $r = 0.79$, equivalent to a 1.31 [0.92–3.67] -fold change). All other genes (COL1A1, MMP3, MMP9, and MMP15) were not significantly different between trained and untrained legs when both age groups were jointly analysed.

Age-related differences in the response of metallopeptidases and collagens to resistance exercise

Due to the PCR array showing partly differential responses between young and old subjects, the resistance exercise-induced changes in gene expression were compared between the two age groups. The individual changes in gene expression separated by age group are shown in Figure 1.The changes in the expression of MMP3 (young: 1.71 [0.14–37.39] -fold change, old: 0.56 [0.02–11.16] -fold change, $Z = -0.74$, $p = 0.556$, $r = -0.24$) did not differ significantly between age groups. While real-time quantitative RT-PCR confirmed the exercise-induced reduction in MMP15 gene expression in the subgroup of older subjects (untrained leg: 2.86 [2.23–3.11], trained leg: 1.83 [1.19–2.86], $Z = -2.02$, $p = 0.043$, $r = -0.90$), differences between age groups failed to reach statistical significance (young: 1.00 [0.72–1.59] -fold change, old: 0.64 [0.54–0.98] -fold change, $Z = -1.98$, $p = 0.056$, $r = -0.63$). Finally, MMP9 gene expression increased in young while decreasing in old participants (young: 9.72 [0.31–18.95] -fold change, old: 0.20 [0.05–1.67] -fold change, $Z = -2.19$, $p = 0.032$, $r = -0.69$) confirming the results from the PCR array. No further age-related differences in training-induced gene expression for COL1A1, COL7A1, and ADAMTS1 were found.

Association between strength level and gene expression changes

Correlational analyses were performed to test potential associations between genes that were regulated by resistance training, either generally (COL7A1, ADAMTS1) or differentially between age groups (MMP9, MMP15), and the subjects' baseline strength level (1-RM measures). Changes in COL7A1 gene expression were strongly negatively correlated to relative leg extension 1-RM ($\rho = -0.705$, $p = 0.023$). In contrast, changes in ADAMTS1 ($\rho = -0.012$, $p = 0.973$), MMP15 ($\rho = 0.456$, $p = 0.185$), and MMP9 ($\rho = 0.401$, $p = 0.250$), were not significantly correlated to relative leg extension 1-RM (Figure 2). None of the

changes in gene expression was associated with relative 1-RM leg press (data not shown).

Discussion

The present study aimed to explore whether an acute bout of resistance exercise would differentially affect muscular ECM gene expression in young and old subjects. Screening for several genes involved in ECM organization, development and degradation we identified some matrix metallopeptidases (MMP3, MMP9, MMP15), ADAM metallopeptidases (ADAMTS1, ADAMTS8), collagens (COL7A1, COL1A1), integrins (ITGB3, ITGAL), laminins (LAMB3, LAMA1), and other cell adhesion molecules (CD44, CDH1, SELE, SELL, CTNND2, SPP1) as candidates for being selectively down- or upregulated (>1.5-fold change) in either young or older subjects. Of the six genes validated on individual samples, COL7A1 gene expression decreased while ADMTS1 gene expression increased in response to resistance exercise irrespective of age. Most interestingly, especially MMPs were affected by exercise in an age-associated manner as MMP9 gene expression increased in young and decreased in older subjects while MMP15 was downregulated in older subjects only.

The collagen superfamily with its 28 different isoforms represents the main structural component of the ECM (Ricard-Blum, 2011), whereby in mature muscles collagens are found in the interstitial matrix (COL I, COL III, COL V, XI, XII, XIV, XV, XVIII), in ECM microfibrils (COL VI) and the basal lamina (COL IV, VI, XV, XVIII) (Murphy & Ohlendieck, 2016). The PCR array used in this study comprised 12 different collagen genes, capturing most of the above-mentioned muscular isoforms with the exception of COL III and XVIII. Genes encoding for COL VII, VIII and XVI have been determined as well. All collagens present on the array were detectable by real time PCR (Ct values between 24.5 and 33.6, see supplementary file 1), whereby the genes COL15A1, COL4A2, and COL6A2 showed the highest and COL7A1, COL8A, and COL11A1 the lowest expression levels across the four conditions, and only COL1A1 and COL7A1 seemed to be responsive to resistance training in young and/or old individuals. Therefore, we decided to focus on these two collagens in the validation measurements.

On the individual samples, the up-regulation of COL1A1 found in the PCR array data 6 h after the resistance exercise could not be confirmed. Although the statistical power of this pilot study may have been insufficient to detect potential minor changes, our results are in line with another study showing that the expression of COL1A1 is unchanged 3 h as well as 2 days after a single bout of lengthening contractions, but increases about 6-fold at 27 days post exercise (Hyldahl et al., 2015). Furthermore, enhancements of COL1A1 mRNA levels in skeletal muscle have been detected after 2 and 6 weeks of rehabilitation following a 2-week immobilization period in elderly men, but only when the resistance training was combined with the administration of growth hormone (Takahashi, Hoshino, Kushida, & Inoue, 1995). Feeding comprises another potentially anabolic stimulus and it has been shown that overfeeding for 28 days results in increased COL1A1 mRNA expression levels in skeletal muscles of young healthy individuals (Tam, Chaudhuri, Hutchison, Samocha-Bonet, & Heilbronn, 2017). Taken together, it appears that COL1A1 gene expression can be induced by resistance training (or other anabolic stimuli), but this upregulation occurs only several weeks after training.

In contrast to COL1A1, COL7A1 gene expression was decreased 6 h after the resistance exercise bout. This effect was detected by the PCR array and confirmed on individual samples, whereby a higher relative 1-RM was associated with a higher reduction in COL7A1 mRNA levels. Collagen type VII is a major structural component of anchoring fibrils, found immediately beneath the lamina densa of many epithelia (Nystrom et al., 2013). Genetic mutations in the COL7A1 gene and the resulting alterations in the morphology and numbers of anchoring fibrils are responsible for various dystrophic forms of epidermolysis bullosa (Kuttner et al., 2013). The loss of collagen VII in dermal fibroblasts has a global impact on the cellular microenvironment and is associated with proteome alterations such as a decrease in basement membrane components and an increase in dermal matrix proteins, TGF-β and metalloproteases (Kuttner et al., 2013). Interestingly, COL7A1 expression in skin increases with age (Glass et al., 2013). Besides the generally high expression of COL7A1 in skin tissue, low amounts of mRNA have also been detected in skeletal muscle tissue (Uhlen et al., 2015). Our results indicate that COL7A1 may be involved in the resistance exercise-induced remodelling of the muscular extracellular matrix, but further studies are required to elucidate the underlying mechanisms.

It is well known that MMPs, particularly gelatinase A (MMP2) and gelatinase B (MMP9) play an important role in the adaptive modifications of skeletal muscle induced by physical exercise (Lo Presti, Hopps, & Caimi, 2017). One of the most interesting findings of our study was the age-specific response of MMP9 to the exercise stimulus: the MMP9 mRNA

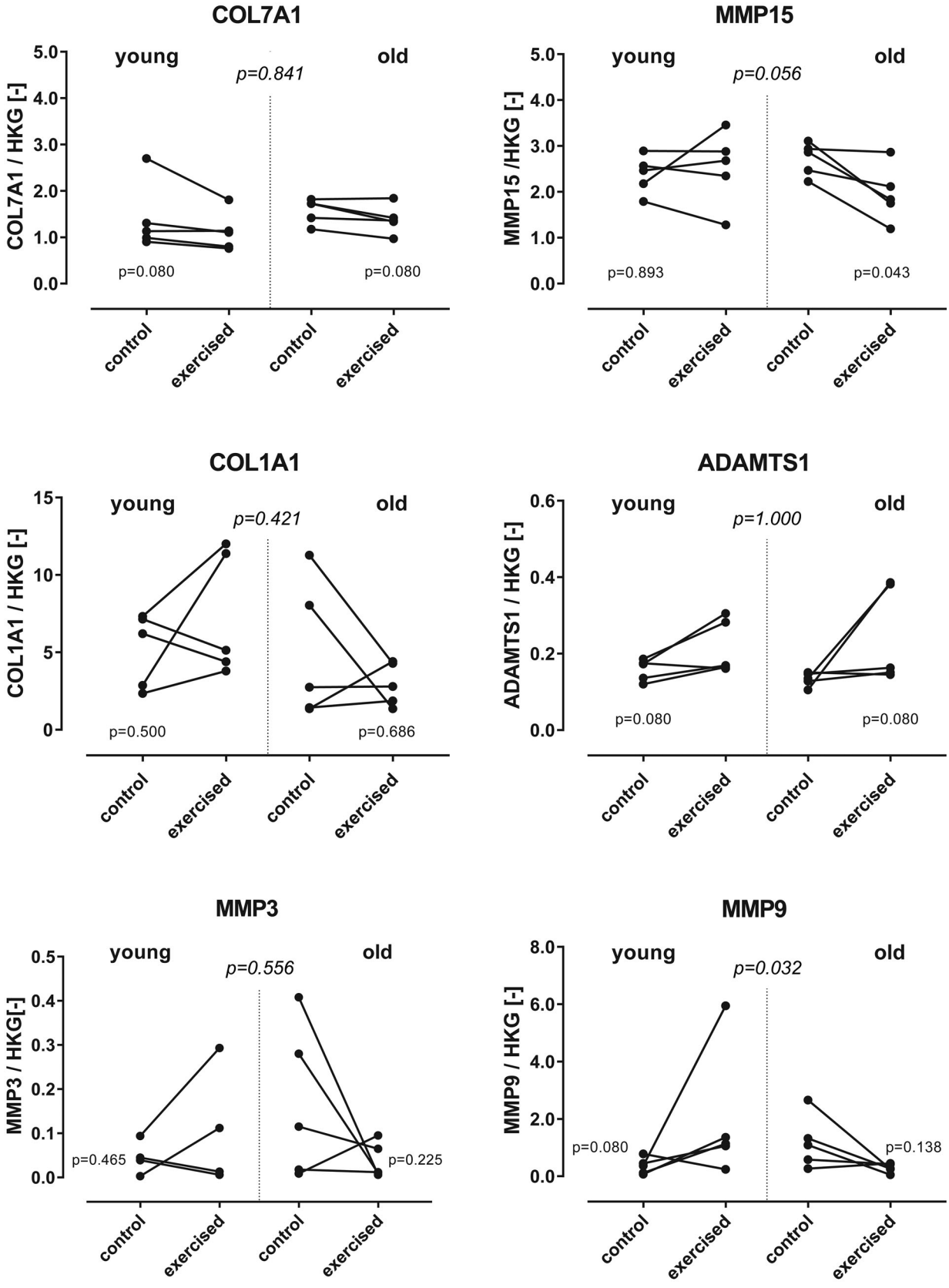

Figure 1. Individual changes in gene expression of genes to be selectively altered in response to resistance exercise in either young or old subjects [COL7A1 (collagen type VII alpha 1), MMP 15 (matrix metallopeptidase 15), COL1A1 (collagen type I alpha 1), ADAMTS1 (ADAM metallopeptidase with thrombospondin type 1 motif 1)] or differentially between age groups [MMP3 (matrix metallopeptidase 3) and MMP9 (matrix metallopeptidase 9)]. Overall *p*-values refer to differences in –fold changes between young and old subjects as tested by Mann-Whitney-U tests, whereas *p*-values presented separately for each age group are based on Wilcoxon tests comparing gene expression of untrained and trained legs ($n = 5$ per group).

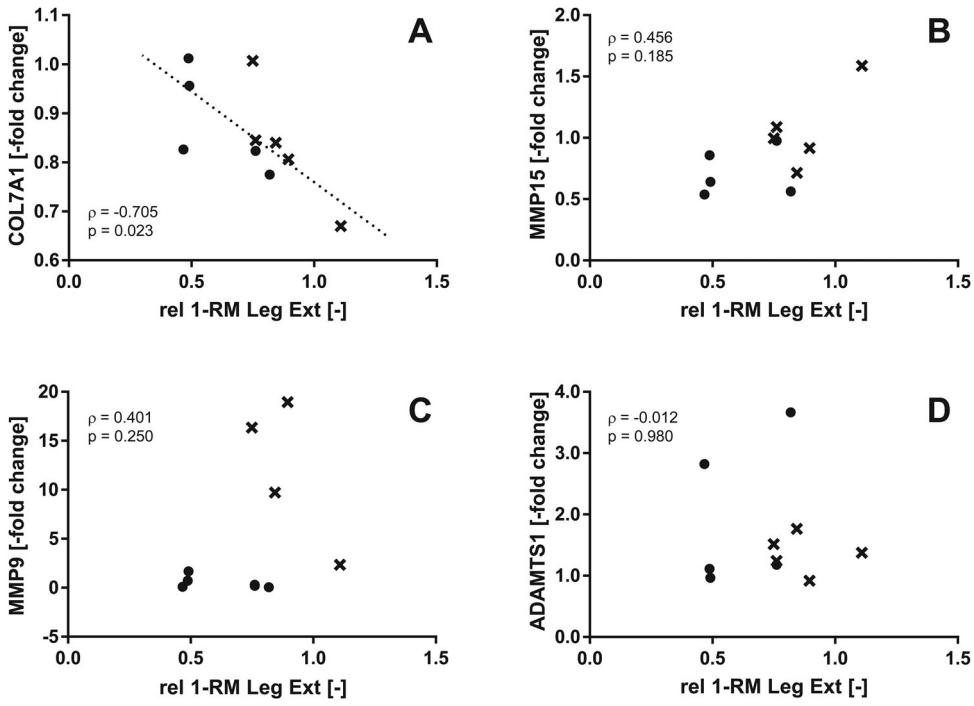

Figure 2. Correlations between the relative 1-repetition maximum for leg extension at baseline and the resistance exercise induced -fold changes of COL7A1 (A), MMP15 (B), MMP9 (C), and ADAMTS1 (D). Solid dots represent old and crosses mark young participants. Spearman correlation coefficients (ρ) and p-values are provided. If the p-value was below 0.05, the line of best fit is displayed as well, $n = 10$.

expression was increased in young but decreased in older subjects. These results are in line with a study in young recreationally active men, where MMP9 mRNA and protein levels were elevated 3 h post knee extension exercises (Patel et al., 2017). To our knowledge no other study so far has reported the exercise-associated MMP9 response in older healthy subjects, but in several models of muscular dystrophy the pharmacological inhibition of MMP9 has been suggested to improve skeletal muscle structure and function and to reduce muscle injury, inflammation and fibre necrosis (Li, Mittal, Makonchuk, Bhatnagar, & Kumar, 2009). However, at later stages of the disease the inhibition of MMP9 may lead to accumulation of fibroadipose tissue and reduced muscle strength (Shiba et al., 2015) showing the multifaceted role of MMP9. Studies performed to investigate the inflammatory processes triggered by resistance exercise may help to interpret the downregulation of MMP9 found in older subjects. One particularly interesting study has suggested that muscles of old adults could be "primed" for a stress response. Such priming is indicated by higher baseline levels of STAT3, NF-κB and HSP70 in muscles of old adults, which may increase the sensitivity to cellular stress and promote inflammatory reactions (Thalacker-Mercer, Dell'Italia, Cui, Cross, & Bamman, 2010). The close connection of the ECM to inflammatory pathways is also evident as the induction of ADAMTS1 gene expression observed in our study could be caused by the infiltration of immune cells to sites of exercise-induced muscle injury. ADAMTS1 functions as an extracellular signal that promotes satellite cell activation and muscle regeneration, at least in young mice (Du et al., 2017).

In contrast to the gelatinase MMP9, MMP15 (alias MT2-MMP) belongs to the family of membrane-type metalloproteinases which suggests that these proteins are expressed at the cell surface rather than secreted (Page-McCaw, Ewald, & Werb, 2007). Although its physiological function is not clearly described, MMP15 has been associated with the formation of tubuli during angiogenesis (Lafleur, Handsley, Knauper, Murphy, & Edwards, 2002) and with tumour progression as it might degrade adherens junction proteins thereby promoting epithelial-mesenchymal transition (Liu et al., 2016). However, its role in skeletal muscle remodelling has not been described so far and needs to be confirmed in future studies.

Strengths and limitations

The major strength of the current study is the comprehensive investigation of various genes involved in extracellular matrix remodelling of muscle tissue

caused by a single bout of resistance exercise in both young and older subjects. With this approach we identified the involvement of some genes that have not been described earlier in this context such as COL7A1 and MMP15. By shifting the focus to the ECM as an interesting contributor to exercise-induced adaptations, the study helps to better understand the age-specific responses of skeletal muscles to physical exercise. However, the study involved only a small number of (male) participants, which limits statistical power and may lead to effects of smaller magnitude being missed. A post hoc power analysis using the data for COL7A1 as an example revealed that the within-subject effect (trained vs untrained leg) was very large ($\eta_p^2 = 0.48$) resulting in a probability of 99% to observe this effect with 10 participants, but 56 subjects would have been necessary to achieve a power of 80% for a hypothetical medium between-subject effect (age) of $\eta_p^2 = 0.13$. Furthermore, the pooling of samples for the PCR array, which has been done in an effort to reduce biological variation, makes substantive differences easier to find with a higher accuracy (Kendziorski, Irizarry, Chen, Haag, & Gould, 2005), but reduces the chance to detect small changes. As such, the study can only be seen as a starting point for further investigation.

Another limitation could be seen in collecting only one muscle biopsy 6 h after the acute exercise bout rather than sampling more frequently, thereby limiting the description of the presumably dynamic process of ECM remodelling. The rationale for selecting 6 h as sampling time point was based on the intention to avoid early inflammatory processes occurring between 0 and 4 h post exercise as outlined by Peake et al. (2017), but to capture mRNA changes in "transitional matrix genes" before these changes could be seen on the protein level, e.g. for tenascin-C at the 24 h time point (Sorensen, Skousen, Holland, Williams, & Hyldahl, 2018). Using this approach, we were able to detect evidence of rather early ECM remodelling, but more measurements points are required to obtain a comprehensive picture of the time kinetics of changes in ECM gene expression following exercise. Finally, it must be stressed that muscle biopsies contain various cell types (skeletal muscle cells, immune cells, fibroblasts, …), which makes it difficult to identify the exact source of a gene product and complicates mechanistic interpretations, especially when considering the crosstalk mechanisms which have been extensively shown for skeletal muscle and immune cells (Peake et al., 2017), but also between myogenic precursor cells and fibroblasts (Fry, Kirby, Kosmac, McCarthy, & Peterson, 2017).

Conclusion & future perspectives

The results of this pilot study indicate that acute resistance exercise induces or represses ECM gene expression in skeletal muscle in an age-specific manner. In particular, the activity of genes encoding several matrix metalloproteinases (MMP3, MMP9, and MMP15) may be selectively downregulated in older subjects, which is of potential relevance for the pathogenesis of muscle fibrosis. As we restricted our evaluation to MMPs and collagens, the expression and functional implications of other genes potentially involved in ECM remodelling, such as SPP1 or CTNND, need to be thoroughly tested. Definitely, larger sample sizes would help to confirm the observed age-associated differences in the response to acute exercise. Further research to be performed in our laboratory will expand this area of research by testing different exercise protocols (e.g. eccentric, concentric contractions of different intensities) in acute exercise and chronic training studies.

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Supplemental data

Supplemental data for this article can be accessed here (https://doi.org/10.1080/17461391.2018.1526974).

List of abbreviations

1-RM: One repetition maximum; **ACTB:** actin beta; **ADAMTS1:** ADAM metallopeptidase with thrombospondin type 1 motif 1; **BMI:** body mass index; **COL7A1:** collagen type VII alpha 1; **ECM**: extracellular matrix; **GAPDH:** glyceraldehyde-3-

phosphate dehydrogenase; **GUSB**: glucuronidase beta; **HAS1:** hyaluronan synthase 1; **MMP3:** matrix metallopeptidase 3; **MMP9:** matrix metallopeptidase 9; **MMP15:** matrix metallopeptidase 15; **RPLP0**: ribosomal protein lateral stalk subunit P0.

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