DiVA
http://www.diva-portal.org

This is the published version of a paper published in *The journals of gerontology. Series A, Biological sciences and medical sciences.*

Citation for the original published paper (version of record):

Skoglund, E., Gronholdt-Klein, M., Rullman, E., Thornell, L-E., Strömberg, A. et al. (2020)
Longitudinal Muscle and Myocellular Changes in Community-Dwelling Men Over Two Decades of Successful Aging: The ULSAM Cohort Revisited
The journals of gerontology. Series A, Biological sciences and medical sciences, 75(4): 654-663
https://doi.org/10.1093/gerona/glz068

Access to the published version may require subscription.

N.B. When citing this work, cite the original published paper.

Permanent link to this version:
http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-173838

THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2020, Vol. 75, No. 4, 654–663
doi:10.1093/gerona/glz068
Advance Access publication April 19, 2019

OXFORD

Original Article

Longitudinal Muscle and Myocellular Changes in Community-Dwelling Men Over Two Decades of Successful Aging—The ULSAM Cohort Revisited

Elisabeth Skoglund, MD,[1,2,3,†,*] Max Grönholdt-Klein, MD,[4,†] Eric Rullman, MD, PhD,[1,†] Lars Eric Thornell, MD, PhD,[3] Anna Strömberg, MD, PhD,[1] Anu Hedman, MD, PhD,[5] Tommy Cederholm, MD, PhD,[2] Brun Ulfhake, MD, PhD,[4,‡] and Thomas Gustafsson, MD, PhD[1,‡]

[1]Department of Laboratory Medicine, Division of Clinical Physiology, Karolinska Institutet, and Unit of Clinical Physiology, Karolinska University Hospital, Stockholm, Sweden. [2]Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University, Sweden. [3]Department of Integrative Medical Biology, Umeå University, Sweden. [4]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden. [5]Heart Centre East-Tallinn Central Hospital, Estonia.

[†]Contributed equally.
[‡]Joint senior authors.
*Address correspondence to: Elisabeth Skoglund, MD, Husargatan 3, BMC, Box 564, 751 22 Uppsala, Sweden. E-mail: elisabeth.skoglund@pubcare.uu.se

Received: August 15, 2018; Editorial Decision Date: March 6, 2019

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

Participants of the population-based Uppsala longitudinal study of adult men (ULSAM) cohort reaching more than 88 years of age (survivors, S) were investigated at age 70, 82, and 88–90 and compared at 70 years with non-survivors (NS) not reaching 82 years. Body composition, muscle mass and muscle histology were remarkably stable over 18 years of advanced aging in S. Analysis of genes involved in muscle remodeling showed that S had higher mRNA levels of myogenic differentiation factors (Myogenin, MyoD), embryonic myosin (eMyHC), enzymes involved in regulated breakdown of myofibrillar proteins (Smad2, Trim32, MuRF1,) and NCAM compared with healthy adult men (n = 8). S also had higher mRNA levels of eMyHC, Smad 2, MuRF1 compared with NS. At 88 years, S expressed decreased levels of Myogenin, MyoD, eMyHC, NCAM and Smad2 towards those seen in NS at 70 years. The gene expression pattern of S at 70 years was likely beneficial since they maintained muscle fiber histology and appendicular lean body mass until advanced age. The expression pattern at 88 years may indicate a diminished muscle remodeling coherent with a decline of reinnervation capacity and/or plasticity at advanced age.

Keywords: E3 ligases, Healthy aging, Skeletal muscle, Sarcopenia, Gene expression

Aging is associated with a decline in muscle mass and function. Eventually such processes may progress to sarcopenia, which consequently leads to frailty and disability and is associated with a range of morbidities (1). From human cross-sectional studies, the decrease in muscle mass has been estimated to be 0.5% per year in men and 0.4% in women, a rate that accelerates at advanced age (0.6%–0.9% per year) (2–5). Although the estimates of the decline of skeletal muscle mass and function vary between studies, the reduction in muscle strength is often even more pronounced than the loss of muscle mass (2,6–11). One reason for the heterogenic loss of muscle mass could be that data are mainly derived from cross-sectional studies comparing age cohorts raised under different conditions. Another aspect is that individuals of identical chronological age could represent different "biological age" (12). Consequently, longitudinal studies assessing individual trajectories of changes in muscle mass and function during aging are warranted (2,13). Even though longitudinal studies also tend to show variable changes in muscle mass and myofiber cross-sectional area (CSA) during aging, loss of muscle strength appears to be a

© The Author(s) 2019. Published by Oxford University Press on behalf of The Gerontological Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

consistent finding irrespective of study design (6–11,14,15). Coherent with findings from small rodents, initial maintenance of reinnervation capacity followed by its successive loss in advanced aging has recently been demonstrated in human skeletal muscle (16) and the ability of human skeletal muscle to remodel after immobilization or disuse is also shown to diminish with aging (17,18). Although some recent successful attempts have been made to define a gene signature of the aging skeletal muscle using bioinformatics tools (19,20), the mechanisms leading to dysregulation of muscle maintenance in the human aged skeletal muscle are still largely unknown and our current understanding stems largely from work done on laboratory animals (21–24). Studies on aged rats have shown signs of activation of proteolysis (via the Ubiquitin Proteasome System) in parallel with alterations in innervation during early aging (21–23,25), suggesting an increased remodeling whereas the activation of Ubiquitin Proteasome System show signs of dysregulation in the oldest muscle (25) in parallel with a decreased capacity to re-innervate vacated myofiber (26). It has also been suggested that maintained proteolytic activity is important during aging for preserved remodeling and quality control of sarcomeric proteins. Indeed, sarcopenia could be accelerated if protein degradation is impaired (27). In humans there is a paucity of studies exploring variances and underlying causes for the different processes leading to sarcopenia and its progression. To address these needs, we have revisited the Uppsala longitudinal study of adult men (ULSAM) to examine factors demonstrated to participate in skeletal muscle remodeling by detailed assessment of individual cases (n = 28 from the upper quartile of survivors [S] in the cohort) over an extended period (from 70 to 88–90 years of age) of advanced aging.

ULSAM is a longitudinal, epidemiological, population-based study where all men born between 1920 and 1924 and living in Uppsala County, Sweden were invited to participate. Initially, 2,322 men accepted the invitation and investigations were made at the ages 50, 60, 70, 77, 82, and 88 years of age. Using health data and muscle biopsies taken at 70 years we have previously reported (28) that both frequency of slow muscle fibers (Type I) and muscle capillarization correlate inversely with arterial pressure and resting heart rate during early aging (50–70 years) in euglycemic cases with essential hypertension. In a second study covering 475 of the cases contributing a muscle biopsy at 70 years, we reported on fiber type distribution based on enzyme activity profiling, muscle capillarization, level of self-assessed physical activity (PA) and insulin sensitivity; in particular, clinical components of the insulin resistance syndrome (IRS) (29). High PA, low BMI associated with a high frequency of Type I fibers and capillarization correlated with low index of biomarkers of IRS while a high frequency of Type II fibers, low muscle capillarization, and PA correlated with markers of IRS in early aging (*idem*). Here we report a longitudinal follow-up of those cases of the 2002 study that reached 90 years covering changes over 18 years of advanced aging with regard to muscle mass and morphology (fiber types and number of satellite cells [SC] per fiber), clinical data, and transcriptional changes in a set of candidate genes involved in muscle remodeling (21–24,30–33). We hypothesized that similar to observations made in small rodent models on sarcopenia there are signs of a higher rate of remodeling (gene expression profile) at early aging (70 years) compared to advanced age (88–90 years) (24,25). Since sarcopenia is closely associated with increased morbidity and death during aging, we also hypothesized that non-survivors (NS) in the ULSAM cohort is more similar to the S when examined at more advanced age than at the age of 70 years. For this purpose, we compared the data at age 70 of the target group of S with corresponding data from a subset of the ULSAM cohort that did not survive until the checkup at age 82. Eight young (25–35 years of age) sedentary men without any medical history (healthy adult men) were used as an independent reference point for gene expression in early adulthood.

Materials and Methods

Study Population

Of the original ULSAM cohort of 2,322 men aged 50 years, 1221 were reexamined at age 70 (1990–1995). At the reexamination, a random sample of 515 men donated a biopsy from m. vastus lateralis. Muscle morphology of these biopsies has been reported in previous studies (28,29). A follow-up at 82 years of age (n = 530 of the original cohort and 226 of the sub-cohort donating a biopsy at 70 years of age) and at 88 years of age (n = 354 of which 158 from those donating a biopsy at 70 years of age) were conducted in 2003–2005 and 2008–2009, respectively. From 28 subjects in this latter group, we successfully obtained a muscle biopsy during 2011–2012 (ie, at an age of 88 to 90 years of age). The selection criterion for inclusion was that the biopsy material from age 70 was still available and intact. This group was used for the longitudinal comparison of muscle biopsy outcomes (Table 1). Given the lifespan expectancy of men born in Sweden between 1920 and 1924, we considered men reaching the age of 88–90 years as successfully aged (ie, being among one of the four S of the cohort). For comparison, we used a random sample of subjects (n = 24) that died before age 82 but who donated a biopsy at 70 years of age (flow diagram in Supplementary Material). Skeletal muscle biopsies taken from eight young sedentary men without any medical history (25–35 years of age) were used as an independent reference point for gene expression in early adulthood. This study complies with the declaration of Helsinki, participants gave their informed consent for participation and this study was approved by the Ethical regional board Uppsala (Dnr: 251/90, Dnr: 02- 605, Dnr: 2007/338 and Dnr: 2010/400, DNR 2006/1232-31/1 and DNR 2010/786-31/3).

Clinical and Laboratory Measurements in the ULSAM Database

The selection of the two study groups (S after 88 years of age and NS at the 82-year follow-up) investigated in the present study was based on the availability of muscle biopsy specimens at 70 years of age rather than a random sample of the larger ULSAM cohort. Therefore, in order to assess if the individuals investigated constituted a representative subset of their respective part of the whole cohort, baseline characteristics form 70 years of age were obtained from the ULSAM database. These data are summarized in Table 1.

Clinical features of the study groups at 70, 82 and 88 years of age are summarized in Table 1 and 2. In addition, in some of the analyses we used muscle morphology data that were partly reported earlier (28,29). Leisure-time PA was assessed with four questions as earlier described (29). Hand grip strength was measured using a Baseline hydraulic hand dynamometer. Both hands were measured three times with 10 seconds rest in between, strongest hand is reported here. Gait speed was assessed during a 10 m walk at comfortable speed. The gait speed in the middle 6 m was recorded.

Dual-Energy X-Ray Absorptiometry

Total lean and fat mass, as well as bone mineral density (BMD, g/cm^2), were measured using dual-energy X-ray absorptiometry (DXA; DPX Prodigy, Lunar corp., Madison, WI) at age 82 and 88. Both extremities

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

Table 1. Baseline Characteristics at 70 Years of Age

	Survivors (n = 187)		Non-survivors (n = 139)			Survivors investigated (n = 28)		Non-survivors investigated (n = 24)	
	Mean	*SD*	Mean	*SD*	*p*-value	Mean	*SD*	Mean	*SD*
Weight	79.5	9.9	82.7	13.4	<.05	78.1	7.6	87.3	10.4
Body mass index (kg/m^2)	26	2.7	26.7	3.8	<.05	25.4	2.3	28.1	3.1
Hip-waist ratio	0.9	0.05	1	0.06	ns	0.94	0.04	1	0.06
Systolic blood pressure (mmHg)	143	17	149	21	<.01	145	17	151	15
Diastolic blood pressure (mmHg)	83	9	84	10	ns	82	8	85	9
Glucose (mmol)	5.6	1	6	1.5	<.01	5.3	0.5	6.3	1.3
Total cholesterol (mmol)	5.9	1	5.8	1	ns	5.9	0.9	5.7	0.7
Muscle Type I fiber (%)	50	15.5	46.6	16.8	ns	49.1	11.6	39.2	16.7
Muscle fiber mean area	5109	1315	5121	1519	ns	5115	1385	5053	1546
Capillaries per fiber	1.5	0.3	1.5	0.4	ns	1.4	0.3	1.6	0.5
Capillaries per mm^2	313	63	312	68	ns	293	57	320	67
Hypertension treament (%)	23		39		.009	18		63	
Smoker (%)	16		28		.02	18		21	
Social group at 50 y (%)									
1	18		13		ns	21		4	
2	44		40			63		38	
3	39		47			16		58	
	101		100			100		100	
Leisure-time physical activity									
Sedentary	1		8		<.01	0		5	
Moderate	31		35			35		45	
Regular	62		52			58		45	
Athletic	6		5			8		5	
	100		100			101		100	
Education at 50 y									
Unknown	4		8			0		13	
≤7 y	58		63		ns	42		71	
8 y	18		17			26		8	
12 y	6		5			16		4	
3 y or more college	8		5			16		4	
Graduate exam	6		2			0		0	
	100		100			100		100	

Note: Baseline characteristics at age 70, mean and when applicable standard deviation (*SD*) for all survivors (survived until the age of >88 y, n varies between 181 and 187 across different variables) and non-survivors (died before the follow-up at age 82, n varies between 124 and 139) and survivors investigated (n varies between 19 and 28 depending on variable) and non-survivors investigated (n varies between 20 and 24) in this study. p-values when comparing survivors and non-survivors, $p < .05$ is considered significant, nonsignificant denoted by ns.

were used in the calculation. The precision of the DXA measurements as calculated by triple measurements in 15 individuals was 1.0% for total lean mass, 1.5% for total fat mass, and 0.8%–1.5% for BMD.

Muscle Biopsies

The percutaneous needle biopsy technique was used to obtain a muscle biopsy from the 28 subjects at age 88–90. Muscle tissue for immunohistochemistry and RNA analysis was snap-frozen in isopentane and stored at −80°C. Skeletal muscle biopsies at age 70 for each individual donating a biopsy at 88–90 as well as for the random sample of subjects (n = 24) were retrieved from the ULSAM cohort freezer repository (−80°C). The procedure to obtain muscle biopsies and the histochemistry protocol at age 70 has been described in detail earlier (28,29).

Sectioning, Immune-Labeling, and Microscopic Analyses of Muscle Biopsies

The inclusion criterion for these analyses was inspection of size of the remaining biopsy at 70 years of age. Tissue sectioning, storage of sections, and immune-labeling have been described in detail elsewhere (34). A list of the used antisera/antibodies including secondary antibodies, dilution, and source are all available in Supplementary Material. Fiber types were identified through staining with antibodies against MyHC isoforms visualized with peroxidase-anti-peroxidase (PAP) staining (35) and a combination of A4.951 and N2.261 was used to assesses the mixed fiber types (I+IIA, IIAX) and Type IIX (Supplementary Figure S1 and Supplementary Table S1). Type IIX fibers were identified by the absence of staining from both antibodies. The mab NCL-Merosin against laminin alpha 2 chain was used to identify the myofiber basal lamina (Supplementary Table S1). Analyses of fiber types at the age of 70 were made using Photoshop and fiber types and CSA at the age of 88–90 were conducted with Leica Qwin plus program. Because of tissue integrity limitations, the minimum number of fibers per case used for fiber type and CSA measurement was 45. The areas in the muscle specimen cross-section to be counted were selected randomly. Compromised fibers were excluded. Only 16 of the biopsies at the age of 70 had sufficient quality for fiber type analysis and none of the biopsies could be used for

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

new measurements of fiber area (the fibers were squished, shrunken or affected by ice crystals). To allow comparison with earlier data reported by Hedman and colleagues (28,29), we tested whether the percentage of the different fiber types (by myosin immunohistochemistry) differed from those reported by Hedman and colleagues for the same subjects at age 70 (using enzyme histochemistry; n = 12). This analysis revealed no significant difference (Supplementary Figure S2) between current data and our earlier report (28,29). To further minimize systematical methodological errors, the area of Type I and Type II fibers was compared using the ratio Type II:Type I. When data were compared with our earlier data (28,29), the fibers were needed to be grouped in to Type I fibers (Type I and Type I+IIA) and Type II fibers (Type IIA, IIAX, and IIX). The multiple marker method (34) was used for staining of SC (Supplementary Figure S3a–c; Supplementary Table S1). More than 100 fibers were counted to obtain SC and myonuclei per fiber. All nuclei that were in the fiber but separated from the lamina were counted as internal nuclei. Areas with obliquely or longitudinally cut fibers, not intact fibers, and poor-quality fibers were excluded. Because five biopsies at age 70 had sufficient quality for SC and myonuclei quantification, only five cases were included in this longitudinal comparison (Supplementary Figure S3a–c and S4).

RNA Extraction and qPCR

Total RNA was prepared by the Trizol method (Invitrogen, Life Technologies) using zirconia beads in a bullet blender and quantified spectrophotometrically by absorbance at 260 nm. RNA integrity (RQI; RNA Quality Index 1–10) was measured with the Experion RNA StdSens & HighSens Kit, the latter for samples with smaller amount of extracted RNA (Bio-Rad Laboratories, Inc.). RNA-concentration was re-measured with PicoDrop (Picopet01, Picodrop Ltd., Cambridge, United Kingdom). Reverse transcription was performed with Applied Biosystems High Capacity cDNA Reverse Transcription Kit as described in detail in Supplementary Material.

Primers were designed using NCBI software Primer-BLAST (nucleotide sequence and accession number in Supplementary Material). Amplicons were controlled with respect to length and number of products by qPCR with melt curve as well as agarose gel electrophoresis using ethidium bromide as stain. For details on PCR-reactions and primers used, see protocol and Table S2 in Supplementary Material. Since there was a substantial variation in RNA integrity measured as RQI across samples (see below) and some of the gene expression changes correlated with RNA integrity, three different genes (GAPDH, β-Actin and GPx1) were tested as internal controls to reduce the impact of RNA integrity between cases. All three gene products generated similar results (Supplementary Figure S5). Because GPx1 has been used in our small rodent studies, CT values were normalized (ΔCT) with the endogenous control GPx1. Correction for intra-experimental-efficiency was conducted using a carry-over triplicate on each plate from an aliquoted cDNA-pool for identical freeze–thaw cycles.

Muscle tissue samples sufficient for extraction and mRNA analyses were available for 25 subjects at both age 70 and age 88–90 and for 24 NS. As expected for long-term stored materials, RQI values varied between samples (range: 1.9 and 9). Normalization (ΔCT) with GPx1 removed most of the correlations between CT and RQI evident in the samples with low RQI. Furthermore, as a precaution the RQI was included in the statistical modeling described below using ΔCT values only and, finally, PCR result constrained to samples with RQI > 5 are presented in Supplementary Material (Supplementary Tables S3–S5). These results show that the outcome of the analyses including all samples is similar to that of the smaller sample of cases with high RNA integrity.

Statistical Analyses and Models

Paired and unpaired two-tailed t-tests were used for group-wise and pair-wise comparisons with regard to baseline characteristics, where a p-value of <.05 was considered significant. Principal Component Analysis was performed on R version 3.3.3. PCA was used (a) to explore correlations of measures of anthropomorphic data at 70, 82, and 88 years of age with DXA measurements at 82 and 88 years of age, (b) to identify clusters of co-varying genes at 70 and 88–90 years of age, and (c) to visualize correlations of gene expression and histological data at 70 years of age. Since there was a substantial variation in RNA integrity measured as RQI across (see above), we analyzed gene expression using a generalized linear model, controlling for differences in RNA integrity to minimize the confounding effect of this variability: Group ~RQI+ΔCT, where "group" denotes either S or NS at 70 years of age, or higher or lower functional capacity at 88 years of age. As expression of multiple genes was tested, compensation for multiple hypothesis tests was carried out by calculating false discovery rate where a false discovery rate of less than 5% was considered significant (36). Wilcoxon test was used to differentiate between fiber type distributions.

Results

Characteristics of the ULSAM Men at Age 70 and at 88–90 Years

The median survival age of the ULSAM cohort was 83 years, which coincides closely with the expected median lifespan of men in the whole country in 2015 (81.5 years; source Central Agency of Statistics, Sweden). Approximately 28% were still alive at the time of the second muscle biopsy; Figure 1. Figure 1 shows that the survival curve for those that donated a biopsy at age 70 (n = 515) adhered to that of the whole ULSAM cohort.

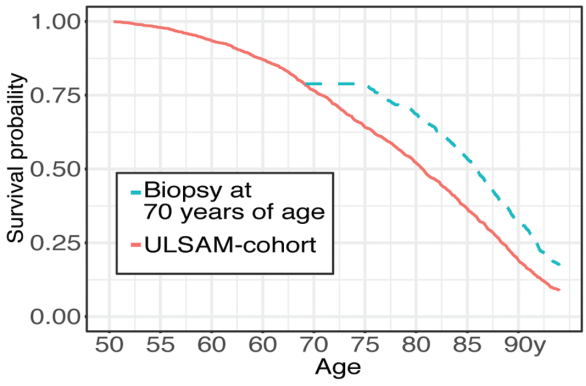

Figure 1. Survival of the Uppsala longitudinal study of adult men (ULSAM) cohort. The survival (1970–2015; 50 to 95 years of age) for the ULSAM cohort, indicated by red-line. The subjects of the current study were recruited from this latter group of survivors. The corresponding survival data for the sub-group that contributed a muscle biopsy at 70 years of age follow-up indicated blue dotted line. The sub-group that contributed a muscle biopsy had a better than average survival for the 5 years following the investigation at 70 years of age (p < .01), thereafter the mortality rate was similar as in the group as a whole. At 90 years of age, 28% of the group was still alive.

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

Clinical, laboratory, and demographic data at the 70, 82, and 88 years' follow-ups of S and of the NS (deceased before the follow-up at 82 years), as well as the cases included in this study for analyses of muscle biopsy, are given in Table 1 and 2. Data in Table 1 describes the groups of S and NS donating a muscle biopsy at age 70. As expected, there were differences between the S and NS with regards to body composition, level of PA and several measures of morbidity, including tobacco use, fasting blood glucose and prevalence of hypertension where S displayed a more favorable profile (Table 1). The cause of death among the NS is shown in Supplementary Figure S6a and b, including the subset also studied regarding gene expression and muscle histology. The main diagnosis group is cardiovascular diseases (43% of the whole cohort of NS; 48% of the subset used in this study), which is expected among men born early-on in the 20th century in Sweden (Supplementary Figure S6a and b).

Muscle Mass, Muscle Strength, and Morphology of M. Vastus Lateralis at age 70 and 88–90

There was a significant decrease in self-reported leisure-time PA over time among S (Table 2). Appendicular muscle mass measured by DXA and body mass index remained remarkably stable although there was a slight decrease in BMI between 82 and 88 years ($p < .01$) (Table 2). In addition, 0.1% per year of appendicular muscle mass/height^2 and 0.3%–0.4% of total lean mass per year were lost between ages 82–88 in S investigated with muscle biopsy as well as in all S.

Staining for fiber types revealed that when fibers were grouped into either Type I, Type I+IIA or Type II fiber (IIA, the mixed fiber Type IIA+IIX and IIX), there was an increase of Type I+IIA hybrid fiber frequency ($p < .05$) and a decrease in Type II fiber frequency ($p < .05$) between age 70 and 88–90 (Figure 2a). All individuals had the mixed (hybrid) fiber Type I + IIA at age 88–90 with an individual variation between 1% and 19% (Figure 2b). To further relate changes in fiber size with age, histological data from the ULSAM database were used. CSA of Type I fibers was larger than Type II fibers at both 70 and 88–90, and this difference tended to increase with age (Figure 2c). When we compared frequency and CSA of Type I and Type II fibers of S with NS (using data from Hedman and colleagues) (28,29) no significant difference in either Type I fiber frequency or CSA (Figure 2d and e). In a small number of cases ($n = 5$), the number of SC and myonuclei could be assessed (Supplementary Figure S7a–c). The mean number of SC per fiber decreased from 70 to 88–90 in three out of five individuals and the mean in the five individuals decreased from 0.076 ($SD \pm 0.03$) SC per fiber to 0.057 ($SD \pm 0.01$) SC per fiber. The number of nuclei per fiber and internal nuclei per fiber decreased in four out of five cases.

Changes in the Expression of mRNA Transcripts Between the Age of 70 and 88–90

In total, the transcription levels of 14 genes (Figure 3a) were analyzed at ages 70 and 88–90 in S ($n = 25$) (see Materials and Method), at age 70 ($n = 24$) among NS (Figure 3b), and in a reference group of adult healthy adult men (age 25–35 years; $n = 8$; Figure 3a). At 70 years of age, the S had greater mRNA levels of the myogenic differentiation factor (MDF) Myogenin, MyoD, embryonic myosin (eMyHC), NCAM, Smad 2 and the two E3 ligases Trim 32 and MuRF1 compared to the adult reference group (Figure 3a) while Smad3 was lower at age 70 and the level of IGF-1Ec (37) mRNA was markedly decreased (Figure 3a). Longitudinal changes in transcript abundance between 70 years of age and 88–90 years of age were evident for two of the MDFs (myogenin and MyoD) but not MRF4, and for eMyHC, NCAM, Smad2 (all higher at age 70) and

Table 2. Characteristics of Survivors at 82 and 88 Years of Age

	Survivors at 82 ($n = 138$)	Survivors at 88 ($n = 138$)	p-value	Survivors Investigated at 82 ($n = 28$)	Survivors Investigated at 88 ($n = 28$)
Body mass index (kg/m^2)	26.2	25.7	<.001	25.6	25.3
Systolic blood pressure (mmHg)	144	147	ns	144	147
Diastolic blood pressure (mmHg)	81	78	<.001	80	78
Mini mental state examination	27.9	27.1	<.05	28.9	28.5
DXA total lean mass (g)	52,379	51,585	<.0001	53,566	52,713
DXA total fat mass (g)	22,534	22,167	ns	20,290	20,594
DXA appendicular lean mass/height^2	7,515	7524	ns	7,645	7,596
Gait speed m/s		1.36			1.37
Handgrip (strongest hand) (kg)		29.9			32.9
Appendicular lean mass/height^2 < 7,230 g (%)	32	32		28	25
Handgrip <30 kg (%)		45			39
Gait speed < 0.8 m/s (%)		3			-
Sarcopenia (1 + 2 OR 3) (%)		14			18
Leisure-time physical activity (%)					
Sedentary	9	23	<.001	0	14
Moderate	38	28		39	21
Regular	46	22		54	32
Athletic	4	2		7	4
NA	3	24		0	29
	100	99		100	100

Note: Characteristics of survivors (survived until the age of >88) at 82 and 88 years of age, mean values. At age 70, 187 individuals were examined, at age 82 138 individuals were examined ($n = 134–138$ depending on variable) and at age 88 138 individuals were examined ($n = 119–138$ depending on variable). Survivors investigated in this study ($n = 28$ at all variables). p-values denote two-sided paired t-tests for continuous variables and chi-square test for leisure-time physical activity. Prevalence of sarcopenia is based on subject fulfilling the criteria of low appendicular lean mass and either of low gait speed or handgrip strength.

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

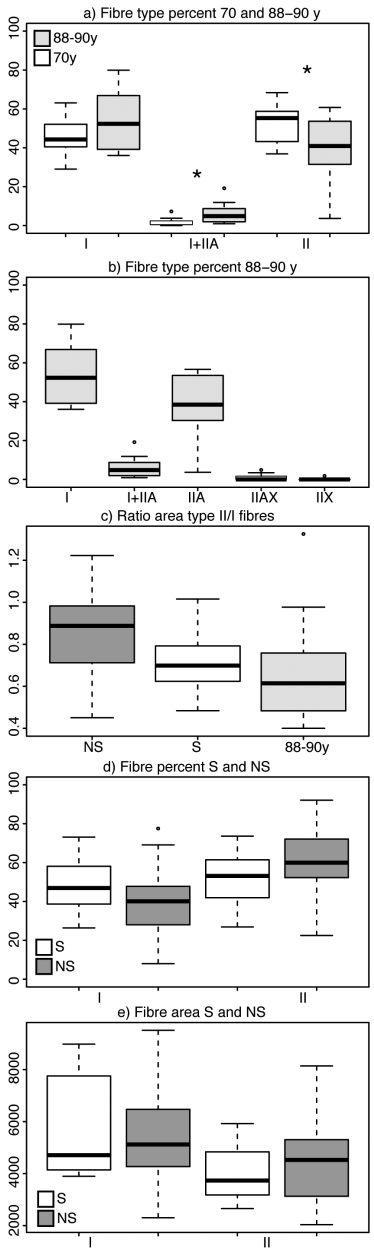

Figure 2. Fiber types, distribution and fiber areas at age 70 and 88–90 (**a**) Percentage of Type I, Type I+IIA and Type II fibers at the age of 70 and 88–90 using immunohistochemistry. Type II includes fiber Type IIA, the mixed fiber Type IIA+IIX, and IIX (n = 16). (**b**) Percentage of different fiber types at age 88–90 using immunohistochemistry (n = 16). The hybrid fibers expressing both Type IIA and Type IIX were only seen in seven individuals, and Type IIX was only found in one individual. **(c)** The ratio of the area of Type II/ Type I fibers in non-survivors (NS, n = 24), survivors (S, n = 12) at age 70 based on ATPase staining data from Hedman and colleagues (28,29) with new data at the age of 88–90 (n = 12) based on immunohistochemistry. For immunohistochemistry Type I fibers include (Type I and Type I+IIA) and Type II fibers (Type IIA, IIAX and IIX)). (**d**) Fiber type percent of Type I (I) and Type II (II) fibers for S (n = 12) and NS (n = 24) at age 70 using data from Hedman and colleagues (28,29). from ATPase staining. (**e**) Fiber area of Type I (I) and Type II (II) fibers for S (n = 12) and NS (n = 24) at age 70 using data from Hedman and colleagues (28,29). based on ATPase staining. ° indicates extreme values. Statistical testing:*p < .05. Whiskers on the boxplots reflect the highest and lowest datum still within 1.5 IQR of the quartile, more extreme datum are depicted as singular points. NS died before age 82 and S survived until the age of >88.

β-Catenin (higher at age 88–90), but not Smad3, Trim32, and MuRF1 (Figure 3a). In comparison with the NS, the S had higher levels of eMyHC, Smad2, and MuRF1 mRNAs at 70 years of age, that is, those transcripts that dropped in abundance between 70 and 88 years among the S (Figure 3b). In contrast, atrogin, PGC-1α, IGF-1Ea and MRF4 showed no significant difference between any of the groups investigated.

When the analyses were constrained to the smaller sets of sample with RQI > 5 (see Materials and method and Supplementary Tables S3–S5), Atrogin mRNA levels were lower in S at 70 than in healthy adult men (p < .05) and decreased further at age 88 (70 years vs 88 years; p < .05) while PGC1a mRNA increased.

Global Analyses

To further analyze co-variations among the parameters and groups (S at age 70 and 88, NS at age 70), we used Principal Component Analysis of correlations between anthropomorphic measures at age 70 and 82 and between anthropomorphic and DXA measures at 88 years of age, which revealed that all of these measures were remarkably stable over time and that they did not correlate with lean body mass at 88 years of age (Supplementary Figure S8). PCA revealed a high degree of covariance among the histological variables (Supplementary Figure S9a) at 70 years of age. Most notably, fiber areas correlated across fiber types and there was a positive correlation between number of Type I fibers and the various measures of capillarity (data from Hedman and colleagues) (28,29). No clustering of S versus NS was identified. Moreover, PCA revealed little covariance between histological variables and gene expression data at 70 years of age (Supplementary Figure S9b).

There was a high degree of covariance in mRNA expression among the genes investigated (Figure 4a and b). Two distinct clusters of genes could be identified where most of the genes clustered along the first principal component, while MuRF1, Smad2, Atrogin, and MRF4 formed a separate cluster along principal component 2. S and NS did not separate along principal component 1, but NS tended to have higher loading on principal component 2. This finding was also reflected in the gene-by-gene analysis where several of the genes with high loading on principal component 2 were significantly different between the groups (Figure 3b). To explore whether the profile of skeletal muscle gene expression at age 88–90 differed based on physical performance, S were split into two groups based on their results on handgrip strength test (<30 kg) and/or gait speed (≤0.8 m/s) at 88 years of age (1). Fourteen S were classified with low physical function based on either maximal handgrip strength of less than 30 kg or a gait speed of less than 0.8 m/s (1). Subjects with a lower function at 88 years of age differed from high functioning subjects by a lower expression of Myogenin, Smad2, and TRIM32 (p < .05).

Discussion

Early identification of people who are at risk for developing age-related decline in muscle mass and function is important in order to improve these individuals' ability to perform activities of daily living at an older age. Accomplishing this goal will require a better understanding of the mechanism(s) underpinning age-induced muscle dysfunction. Here we assessed a group of men from the population-based ULSAM cohort from age 70 to age 88–90, men who belong to the upper S quartile of the cohort (354 out of 2,322 at age 50). The subjects did not display any major alteration in muscle mass (measured by DXA) or fiber type composition despite a decrease in leisure-time PA between the ages of 70 and 88. These results

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

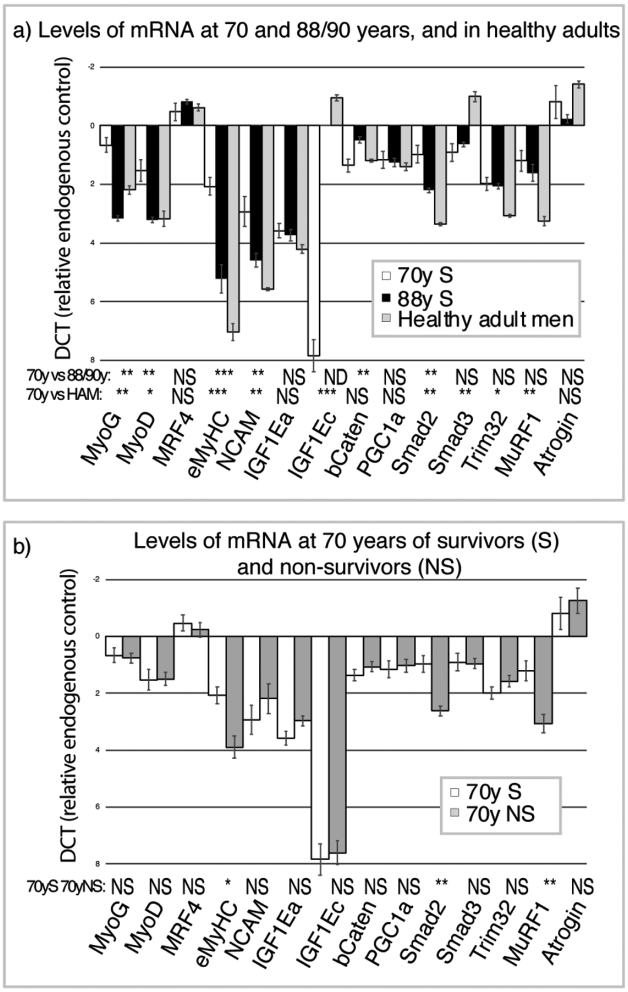

Figure 3. RNA expression at age 70 and 88–90 in relation to healthy young males, and a comparison between survivors and non-survivors at age 70. (**a**) The RNA expression at age 70 (white) at age 88–90 (black) (n = 25) and a reference group of healthy adult men (HAM) aged 25–35 (gray) (n = 8). (**b**) The RNA expression at age 70 in survivors (white) (n = 25) and non-survivors (dark gray) (n = 24). Genes were normalized to GPx1, values in a-b are mean ± SEM. Statistical testing indicated as follows: NS > 0.05, *<0.05, **<0.01, and ***<0.001. Non-survivors are those who died before age 82 and survivors survived until the age of >88.

are consistent with other longitudinal studies of aging women and men (2,6–11,13–15). At age 70, S had greater transcript levels of Myogenin, MyoD, eMyHC, NCAM, Smad 2 and the E3 ligases Trim 32 and MuRF1 in comparison to the young healthy reference group. This gene expression profile became significantly less accentuated at age 88–90, especially in the individuals displaying lower physical function at this age (Figure 4b). At age 70, several genes associated with age-related muscle wasting in both humans and experimental animal models (21–24,30–33) showed an increase in expression in S versus NS.

Longitudinal Study

Although the selection was based on availability of muscle tissue sample at age 70 and 88–90, the cases included appeared to reflect the whole underlying cohort when considering the general health data set (Table 1). The group of men (n = 28) that donated a muscle biopsy at age 88–90, used in the longitudinal analyses, all belong to the long-lived quartile of the ULSAM cohort. Therefore, we define this group as successfully aged. As a group, they show remarkable stability in all clinical measures recorded (Table 2). Despite a decrease in PA between 70 and 88–90 years, lean body mass and other measures did not decrease in a concerted manner or to a substantial degree (Table 2). Consistent with a preserved lean body mass (DXA), histology revealed only small changes with an increase in frequency of Type I+IIA fibers combined with a relative increase of Type I fiber area over Type II fiber area (Figure 2a–e). These observed changes are in agreement with other longitudinal studies (6–11,14,15) and are also consistent with previous observations of muscle fiber histology in aged muscle (13,38). In this study, a loss of appendicular lean mass/height^2 of 0.1% and 0.3%–0.4% of total lean mass per year was observed between ages 82 and 88, a little less than expected from cross-sectional studies (2–5). Studies on aging often have a cross-sectional study design for practical reasons but will then compare age groups born and raised under different conditions; that is revealing cohort effects. Unlike our study, such studies leave out information about the trajectory of changes occurring in individuals as they age. We found that long-lived members of the ULSAM cohort maintained muscle mass and the number of individuals that displayed signs that met the definition of sarcopenia (1) during advanced aging was 14% at 88–90 years of age (Table 1).

Much of what is known today about skeletal muscle development and regulation of myofiber phenotype and metabolic state is derived from experimental rodent studies (21–24) In the present study 14 genes were selected for qPCR analysis (Figures 3a and b, 4a and b). The selection was based on data from experimental animal models (24,25,32,33,39,40), and data from human materials suggesting that these genes are also of relevance in human conditions of muscle hypertrophy and atrophy (30,31). The majority of these genes were expressed at a lower level at 88–90 years of age than at age 70 (Figure 3a), including the myogenic differentiation factors (MyoD and Myogenin), which are needed to complete differentiation from SC to myocyte (22), and eMyHC. Myogenin apparently also serve functions in stabilizing the NMJ (41). Importantly, in comparison with young individuals Myogenin was highly expressed at 70 years of age. This finding suggests that fewer fibers undergo remodeling at 88–90 years of age than at 70 years of age, and at age 70 the increased levels of myogenic differentiation factors likely aid in promoting differentiation of progenitor cells towards mature myocytes. In this study, only five cases could be subjected to an analysis of SC at both 70 years of age and age 88–90, but the data are in line with corresponding data from the literature (42,43) suggesting that in human muscle, SCs decrease with age. An increased level of β-catenin mRNA was observed at age 88 years, which may also suggest an increased stimulus to exit off-springs generated by the self-replicating cycle in a diminishing pool of SC to replenish the myocyte population. The reduced expression of Smad2 mRNA (higher at 70 years of age and lower at 88–90 years of age) is possibly related to its role as a downstream effector of ACTR IIB to increase breakdown/turnover of myofibrillar proteins. This might be of importance for regenerating healthy muscle tissue, but it also sub-serves other functions which deserve further investigation (27,32,44). The tendency for a lower expression of MuRF1 (Figure 3a) matches the downregulation of Smad2 and suggests that the activation of the Ubiquitin Proteasome System observed at age 70 fades-off at advanced age, similar to the pattern observed in small rodents (23–25). This may be related to the concept of collateral reinnervation (induced by failure to maintain innervation), that is, the proposed mechanism underlying fiber type grouping and alteration of motor unit size, which postulates increased remodeling in early aging

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

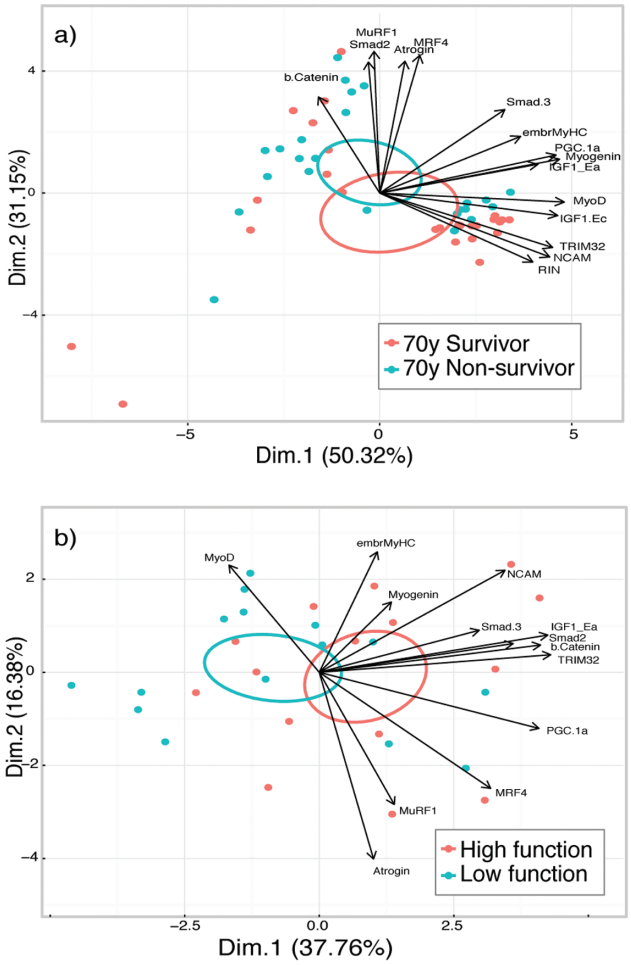

Figure 4. (**a**) PCA analysis of gene expression and RNA integrity at age 70 of survivors (survived until the age of 88) and non-survivors (died before 82 years of age) reveal high degree of covariance among the genes investigated with 82% of the total variance explained by the two first principal components. Two distinct groups of genes can be identified where most of the genes co-vary mainly on the first component but MuRF1, Smad2, Atrogin, and MRF4 form a separate group along principal component 2 (PC2). Survivors and non-survivors do not form clusters and do not separate along principal component 1 (PC1); along PC2, non-survivors have higher loading. This is also reflected in the gene-by-gene analysis where the genes with high loading on PC2 are significantly different between the groups. Finally, there is a substantial variance in RNA integrity (RIN) across samples and several genes, most notably TRIM32 and NCAM co-vary with RNA integrity. (**b**) PCA analysis of gene expression at age 88–90 of survivors with higher (red) versus lower (blue) physical function where low physical function was defined as either maximal handgrip strength of <30 kg or a gait speed of <0.8 m/s. At age 88–90, gene expression displays a substantially lesser degree of covariance than at age 70 with 54% of the variance explained by the first two principal components. Lower physical function is associated with lower loading on PC1 consistent with significantly lower expression of Myogenin, Smad2, and TRIM32 on gene-by-gene analysis.

followed by incapacitation at advanced age, coinciding with observations of an increased number of permanently denervated myofibers (21–23). Support for such events (ie, initially maintained reinnervation capacity followed by successive loss in advanced aging) has recently been demonstrated in human skeletal muscle (16).

Interestingly, we saw no difference in the local expression of the IGF-1 mRNA isoform also produced by the liver (IGF-1Ea), while the "mechano" splice variant of IGF-1 (IGF-1Ec), readily detectable in adult controls, was greatly decreased at 70 years of age and not detectable at 88–90 years of age (Figure 3a and b). These findings are in line with a decreased intrinsic drive for myofiber growth through the IGF-1-Akt-TORC1 pathway with age (23). Such an idea is coherent with observations of a decline in content and activation of SCs in humans as well as a reduced muscle regeneration capacity in response to myofiber injury and disuse (17,45–48). Recently an accumulation rate of 13 somatic mutations/genome/year was observed in human SC, which targeted genes involved in myoblast activity. These mutations were also demonstrated to propagate into the muscle and thereby presumably reduce muscle cell plasticity (49). Thus, the observation that individuals with lower function at 88 years of age differed from higher functioning subjects by a lower expression of Myogenin, Smad2, and TRIM32, may represent individual differences in remaining muscle plasticity

Altogether, the regulation of gene transcription observed at 70 years of age among S should be considered beneficial since these individuals maintained function, muscle fiber histology, and appendicular lean body mass until advanced age. Activation of the atrophy pathways is perhaps needed to achieve a balance in clearance and renewal of sarcomeric proteins when effects of aging infringe on the integrity of the tissue (eg, alterations in innervation, compromised energy production, cellular accumulation of debris, and an accumulation of DNA mutations).

Comparison at 70 Years of Age With NS

Since the cases studied longitudinally were selected based on being alive at 88 years of age and on the availability of biopsy material from 70 years of age remaining in the inventory, we considered it of interest to compare this group with a random sample of those who did not survive until the inventory at 82 years of age. Difference in environmental factors between the long-lived cases studied here and those who died before the expected median lifespan of the ULSAM cohort are coherent with other studies (50). The studied group of 24 NS differed in some aspects from the whole group of NS (n = 139) (Table 1) with a bias towards higher BMI and a lower frequency of Type I fibers in the NS investigated. In our previous studies of the ULSAM cohort, we show that muscle histology associates with morbidities as essential hypertension and biomarkers of the IRS (28,29). The histological examination made here revealed a tendency for a higher frequency of Type I over Type II fibers in S compared with NS at age 70. Combined with data on hypertensive treatment, blood glucose and BMI, being higher among the NS; the results here are consistent with our earlier observations and suggest a link between muscle histology on the one hand, and morbidity and survival, on the other, that deserves further attention. Further differences were evident in the regulation of some of the candidate genes (PCA, Figures 3b and 4a). Gene-by-gene comparison (Figure 3b) revealed a lower expression of both eMyHC and MuRF1 among NS, suggesting that fiber remodeling and myosin turnover was less in NS than S at 70 years of age. With respect to these two genes and the expression of Smad2 mRNA, the NS expression profile was closer to the S profile at age 88–90 than at 70 years of age (see above). Altogether, we conclude that individuals donating a muscle biopsy at 70 in ULSAM and who died between ages 70 and 82 differed in several aspects from the S when compared at 70 years of age.

Concluding Remarks

As shown in this study, men born in the Uppsala County, Sweden between 1920 and 1924 may reach advanced age without signs

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

of sarcopenia. In fact, only 14% satisfied the European Working Group on Sarcopenia in Older People (EWGSOP) recommended criteria of sarcopenia at 88 years of age (1). Many of the transcriptional changes observed in experimental data generated from small rodent models on sarcopenia were also evident in the longitudinal material studied here in early aging (70 years of age) but became less accentuated at an advanced age (88–90 years of age). Thus, the activation of genes like members of the MDF family, eMyHC, genes involved in regulated of proteolysis of sarcomeric proteins and Smad2 may reflect an appropriate adaptive response when aging starts to impact cellular processes and may therefore be considered as beneficial, not detrimental. Gene alteration at advanced age remains unclear but indicates a dysregulation in remodeling coherent with earlier observations of a successive reduced plasticity in aging human skeletal muscle.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

This work was supported by grants from Karolinska Institutet, Magnus Bergwall Foundation, Vetenskapsrådet/Swedish Research Council 2015–02338, Marcus and Marianne Wallenberg Foundation and Department of Integrative Medical Biology at Umeå University.

Acknowledgments

Margareta Enerstedt, Anna-Karin Olofsson, Helene Fischer, Karl Olsson, Tommy Lundberg, and Mona Lindström provided excellent technical assistance.

Conflict of Interest Statement

None reported.

References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al.; European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing*. 2010;39:412–423. doi:10.1093/ageing/afq034
2. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol*. 2012;3:260. doi:10.3389/fphys.2012.00260
3. Tzankoff SP, Norris AH. Effect of muscle mass decrease on age-related BMR changes. *J Appl Physiol Respir Environ Exerc Physiol*. 1977;43:1001–1006. doi:10.1152/jappl.1977.43.6.1001
4. Kyle UG, Genton L, Hans D, et al. Total body mass, fat mass, fat-free mass, and skeletal muscle in older people: cross-sectional differences in 60-year-old persons. *J Am Geriatr Soc*. 2001;49:1633–1640. doi:10.1111/j.1532-5415.2001.49272.x
5. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol (1985)*. 2000;89(1):81–88. doi:10.1152/jappl.2000.89.1.81
6. Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci*. 2001;56:B209–B217. doi:10.1093/gerona/56.5.b209
7. Dey DK, Bosaeus I, Lissner L, Steen B. Changes in body composition and its relation to muscle strength in 75-year-old men and women: a 5-year prospective follow-up study of the NORA cohort in Göteborg, Sweden. *Nutrition*. 2009;25:613–619. doi:10.1016/j.nut.2008.11.023
8. Koster A, Ding J, Stenholm S, et al.; Health ABC study. Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? *J Gerontol A Biol Sci Med Sci*. 2011;66:888–895. doi:10.1093/gerona/glr070
9. Shimokata H, Ando F, Yuki A, Otsuka R. Age-related changes in skeletal muscle mass among community-dwelling Japanese: a 12-year longitudinal study. *Geriatr Gerontol Int*. 2014;14 (Suppl 1):85–92. doi:10.1111/ggi.12219
10. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol (1985)*. 2000;88(4):1321–1326. doi:10.1152/jappl.2000.88.4.1321
11. Reid KF, Pasha E, Doros G, et al. Longitudinal decline of lower extremity muscle power in healthy and mobility-limited older adults: influence of muscle mass, strength, composition, neuromuscular activation and single fiber contractile properties. *Eur J Appl Physiol*. 2014;114:29–39. doi:10.1007/s00421-013-2728-2
12. Sood S, Gallagher IJ, Lunnon K, et al. A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status. *Genome Biol*. 2015;16:185. doi:10.1186/s13059-015-0750-x
13. Grimby G, Evans WJ. Muscle performance and structure in the elderly as studied cross-sectionally and longitudinally. *J Gerontol A Biol Sci Med Sci*. 1995;50 Spec No:17–22. doi:10.1093/gerona/50a.special_issue.17
14. Frontera WR, Reid KF, Phillips EM, et al. Muscle fiber size and function in elderly humans: a longitudinal study. *J Appl Physiol (1985)*. 2008;105(2):637–642. doi:10.1152/japplphysiol.90332.2008
15. Trombetti A, Reid KF, Hars M, et al. Age-associated declines in muscle mass, strength, power, and physical performance: impact on fear of falling and quality of life. *Osteoporos Int*. 2016;27:463–471. doi:10.1007/s00198-015-3236-5
16. Piasecki M, Ireland A, Piasecki J, et al. Failure to expand the motor unit size to compensate for declining motor unit numbers distinguishes sarcopenic from non-sarcopenic older men. *J Physiol*. 2018;596:1627–1637. doi:10.1113/JP275520
17. Suetta C, Frandsen U, Mackey AL, et al. Ageing is associated with diminished muscle re-growth and myogenic precursor cell expansion early after immobility-induced atrophy in human skeletal muscle. *J Physiol*. 2013;591:3789–3804. doi:10.1113/jphysiol.2013.257121
18. Hvid LG, Suetta C, Nielsen JH, et al. Aging impairs the recovery in mechanical muscle function following 4 days of disuse. *Exp Gerontol*. 2014;52:1–8. doi:10.1016/j.exger.2014.01.012
19. Phillips BE, Williams JP, Gustafsson T, et al. Molecular networks of human muscle adaptation to exercise and age. *PLoS Genet*. 2013;9:e1003389. doi:10.1371/journal.pgen.1003389
20. Welle S, Brooks AI, Delehanty JM, et al. Skeletal muscle gene expression profiles in 20-29 year old and 65-71 year old women. *Exp Gerontol*. 2004;39:369–377. doi:10.1016/j.exger.2003.11.011
21. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. *J Physiol*. 2016;594:1965–1978. doi:10.1113/JP270561
22. Tintignac LA, Brenner HR, Rüegg MA. Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting. *Physiol Rev*. 2015;95:809–852. doi:10.1152/physrev.00033.2014
23. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. *Crit Rev Biochem Mol Biol*. 2014;49:59–68. doi:10.3109/10409238.2013.857291
24. Altun M, Grönholdt-Klein, M, Wang L, Ulfhake, B. Cellular degradation machineries in age-related loss of muscle mass (Sarcopenia). In: Nagata T, ed. *Senescence*. InTech; 2012. p. 269–286. doi:10.5772/34274
25. Altun M, Besche HC, Overkleeft HS, et al. Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. *J Biol Chem*. 2010;285(51):39597–39608. doi:10.1074/jbc.M110.129718

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020

26. Aare S, Spendiff S, Vuda M, et al. Failed reinnervation in aging skeletal muscle. *Skelet Muscle*. 2016;6(1):29. doi:10.1186/s13395-016-0101-y
27. Sandri M, Barberi L, Bijlsma AY, et al. Signalling pathways regulating muscle mass in ageing skeletal muscle: the role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology*. 2013;14(3):303–323. doi:10.1007/s10522-013-9432-9
28. Hedman A, Reneland R, Lithell HO. Alterations in skeletal muscle morphology in glucose-tolerant elderly hypertensive men: relationship to development of hypertension and heart rate. *J Hypertens*. 2000;18(5):559–565. doi:10.1097/00004872-200018050-00008
29. Hedman A, Byberg L, Reneland R, Lithell HO. Muscle morphology, self-reported physical activity and insulin resistance syndrome. *Acta Physiol Scand*. 2002;175:325–332. doi:10.1046/j.1365-201X.2002.01000.x
30. Mascher H, Tannerstedt J, Brink-Elfegoun T, Ekblom B, Gustafsson T, Blomstrand E. Repeated resistance exercise training induces different changes in mRNA expression of MAFbx and MuRF-1 in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2008;294:E43–E51. doi:10.1152/ajpendo.00504.2007
31. Fernandez-Gonzalo R, Lundberg TR, Tesch PA. Acute molecular responses in untrained and trained muscle subjected to aerobic and resistance exercise training versus resistance training alone. *Acta Physiol (Oxf)*. 2013;209:283–294. doi:10.1111/apha.12174
32. Sartori R, Milan G, Patron M, et al. Smad2 and 3 transcription factors control muscle mass in adulthood. *Am J Physiol Cell Physiol*. 2009;296:C1248–C1257. doi:10.1152/ajpcell.00104.2009
33. Ibebunjo C, Chick JM, Kendall T, et al. Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. *Mol Cell Biol*. 2013;33:194–212. doi:10.1128/MCB.01036-12
34. Lindström M. New multiple labelling method for improved satellite cell identification in human muscle: application to a cohort of power-lifters and sedentary men. *Histochemistry and Cell Biology*. 2009;132(2):141–157. doi:10.1007/s00418-009-0606-0
35. Sternberger LA. The unlabeled antibody (PAP) method, introduction. *J Histochem Cytochem*. 1979;27:1657. doi:10.1177/27.12.392001
36. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Stat*. 2001;29:1165–1188. doi:10.1214/aos/1013699998
37. Kandalla PK, Goldspink G, Butler-Browne G, Mouly V. Mechano Growth Factor E peptide (MGF-E), derived from an isoform of IGF-1, activates human muscle progenitor cells and induces an increase in their fusion potential at different ages. *Mech Ageing Dev*. 2011;132:154–162. doi:10.1016/j.mad.2011.02.007
38. Lexell J. Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci*. 1995;50 Spec No:11–16.
39. Ge X, McFarlane C, Vajjala A, et al. Smad3 signaling is required for satellite cell function and myogenic differentiation of myoblasts. *Cell Res*. 2011;21:1591–1604. doi:10.1038/cr.2011.72
40. Zhao J, Brault JJ, Schild A, Goldberg AL. Coordinate activation of autophagy and the proteasome pathway by FoxO transcription factor. *Autophagy*. 2008;4:378–380. doi:10.4161/auto.5633
41. Chen HH, Tsai LK, Liao KY, et al. Muscle-restricted nuclear receptor interaction protein knockout causes motor neuron degeneration through down-regulation of myogenin at the neuromuscular junction. *J Cachexia Sarcopenia Muscle*. 2018;9:771–785. doi:10.1002/jcsm.12299
42. Verdijk LB, Snijders T, Drost M, Delhaas T, Kadi F, van Loon LJ. Satellite cells in human skeletal muscle; from birth to old age. *Age (Dordr)*. 2014;36:545–547. doi:10.1007/s11357-013-9583-2
43. Thornell LE. Sarcopenic obesity: satellite cells in the aging muscle. *Curr Opin Clin Nutr Metab Care*. 2011;14:22–27. doi:10.1097/MCO.0b013e3283412260
44. Han HQ, Zhou X, Mitch WE, Goldberg AL. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45:2333–2347. doi:10.1016/j.biocel.2013.05.019
45. Petrella JK, Kim JS, Cross JM, Kosek DJ, Bamman MM. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab*. 2006;291:E937–E946. doi:10.1152/ajpendo.00190.2006
46. Kadi F, Charifi N, Denis C, Lexell J. Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve*. 2004;29:120–127. doi:10.1002/mus.10510
47. Renault V, Thornell LE, Eriksson PO, Butler-Browne G, Mouly V, Thorne LE. Regenerative potential of human skeletal muscle during aging. *Aging Cell*. 2002;1:132–139. doi:10.1046/j.1474-9728.2002.00017.x
48. Petrella JK, Kim JS, Mayhew DL, Cross JM, Bamman MM. Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis. *J Appl Physiol (1985)*. 2008;104(6):1736–1742. doi:10.1152/japplphysiol.01215.2007
49. Franco I, Johansson A, Olsson K, et al. Somatic mutagenesis in satellite cells associates with human skeletal muscle aging. *Nat Commun*. 2018;9:800. doi:10.1038/s41467-018-03244-6
50. Andersen K, Lind L, Ingelsson E, et al. Skeletal muscle morphology and risk of cardiovascular disease in elderly men. *Eur J Prev Cardiol*. 2015;22:231–239. doi:10.1177/2047487313506828

Downloaded from https://academic.oup.com/biomedgerontology/article-abstract/75/4/654/5475293 by Umea universitet user on 03 August 2020