High-abundance mRNAs in human muscle: comparison between young and old

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Welle, Stephen, Kirti Bhatt, and Charles A. Thornton. High-abundance mRNAs in human muscle: comparison between young and old. J Appl Physiol 89: 297–304, 2000.—To gain a better understanding of the potential role of altered gene expression in the diminished muscle function in old age, we performed a broad search for transcripts expressed at quantitatively different levels in younger (21–24 yr) and older (66–77 yr) human vastus lateralis muscle by serial analysis of gene expression (SAGE). Because SAGE was based on RNA pooled from muscle of several different subjects, relative concentrations of selected mRNAs also were determined in individual muscle samples by quantitative RT-PCR. There were 702 SAGE tags detected at least 10 times in one or both mRNA pools, and the detection frequency was different (at P < 0.01) between young and older muscle for 89 of these. The ratio of myosin heavy chain 2a mRNA to myosin heavy chain 1 mRNA was reduced in older muscle. The mRNAs encoding several mitochondrial proteins involved in electron transport (including several subunits of cytochrome-c oxidase and NADH dehydrogenase) and subunits of ATP synthase were ~30% less abundant in older muscle. Several mRNAs encoding enzymes involved in glucose metabolism also were less abundant in older muscle. Analysis of individual samples revealed that the differences suggested by SAGE were not artifacts of atypical gene expression in one or a few individuals. These data suggest that some of the phenotypic changes in senescent muscle may be related to altered gene transcription.

METHODS

Subjects. Sixteen men (8 young, 21–24 yr old; 8 older, 66–77 yr old) were recruited by newspaper advertisements. All subjects were healthy as determined by history, physical examination, and laboratory tests (glucose, electrolytes, thyroid-stimulating hormone, liver enzymes, creatine kinase, creatinine, urea, complete blood count). Neuromuscular function was normal in all subjects. None was engaged in any strenuous exercise program. Written consent was obtained from all subjects after the procedures and risks were explained. The project was approved by the University of Rochester Research Subjects Review Board. The SAGE inventory of the younger muscle has been described elsewhere (28).

Procedures. An important feature of this study is that subjects followed a standardized protocol before donating muscle. Subjects refrained from exercise more strenuous than walking for 3 days before being admitted to the University of Rochester General Clinical Research Center. They were admitted the evening before the muscle biopsy procedure for more stringent control of diet and activity. They received a standard dinner, fasted overnight, and then received a standard breakfast 90 min before the muscle biopsy. The needle biopsy was obtained from the vastus lateralis muscle within a few minutes of anesthetizing the skin and

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mRNAs IN YOUNG AND OLD MUSCLE

muscle. The tissue sample was frozen in liquid nitrogen within 30 s and then stored at −70°C.

Extraction and measurement of total RNA. The frozen muscle was homogenized with a Polytron in 1 ml Tri-Reagent (Molecular Research Center, Cincinnati, OH). The homogenate was centrifuged at 12,000 × g for 15 min, and the aqueous supernatant containing the RNA was transferred to a separate tube for ethanol precipitation. The precipitate was dissolved in RNase-free water (2 µl/mg tissue) and stored at −70°C. The amount of RNA extracted from the tissue was determined by absorbance of ultraviolet light at 260 nm, with background compensation for the absorbance at 320 nm, by using a GeneQuant RNA/DNA calculator (Pharmacia Biotech, Piscataway, NJ).

Polyadenylated RNA. Total RNA (0.4 µg) was applied to a positively charged nylon membrane with a slot-blotting apparatus and hybridized with a 32P-labeled oligo(dT)18 probe as described previously (26). The relative amount of probe bound to each slot was quantified with a PhosphorImager by using the ImageQuant software provided by the manufacturer (Molecular Dynamics, Sunnyvale, CA). The mRNA concentration was determined by comparison of the signal with that of a known amount of human muscle mRNA applied to the same membrane.

SAGE. SAGE was performed as described elsewhere (28) with pooled mRNA from each age group. This method provides a digital profile of gene expression by counting the number of times short (14-base) sequence tags characteristic of each mRNA are detected in a cDNA library.

Quantitative RT-PCR. Very little RNA remained from each individual after samples were pooled for SAGE, not enough to evaluate very many transcripts by Northern blotting or RNase protection. The only method sensitive enough for examination of numerous transcripts was quantitative RT-PCR. This method was used to evaluate the relative levels in individual samples of some of the mRNAs that did not appear (according to SAGE) to be affected by aging (α-actin, myoglobin, creatinine kinase) and some of the mRNAs that appeared to be differentially expressed in younger and older muscle. The latter group included mRNAs encoding myosin heavy chain (MHC) isoforms, for which the RT-PCR assay has been described previously (27). We also focused on mRNAs encoding proteins involved in the mitochondrial electron transport and ATP synthase complexes, because many transcripts in this category appeared to be differentially expressed in young and old muscle. The relative concentrations of mRNAs encoding mitochondrial RNA polymerase and mitochondrial transcription factor A (mtTFA) were determined by quantitative RT-PCR, because these factors are essential for transcription of mitochondrial DNA (mtDNA) (7, 15, 20).

First-strand cDNA was generated with Moloney murine leukemia virus RT, with dT12–18 as the primer. All samples from both age groups (2 µg total RNA/sample) were reverse transcribed at the same time with the same batch of enzyme, in a total volume of 60 µl. Each quantitative PCR assay was done with 1 µl of the RT solution. Assays of many specific cDNAs from replicate RT reactions on the same pool of RNA indicated that the coefficient of variation associated with the RT step was <10%. PCR primers were selected with the assistance of Gene Runner 3.04 software and were based on GenBank nucleotide sequences. The PCR products derived from every primer pair were sequenced to verify that the correct cDNAs were being amplified. Relative quantitation was achieved by using an internal standard in each PCR reaction. The internal standards were produced by deleting several internal bases from the cDNAs (Fig. 1). The standards had the same priming sequences as the cDNAs but could be separated from them by polyacrylamide gel electrophoresis. Each PCR tube contained only a single primer pair and a single internal standard, because variability was greater when we attempted to quantify more than one type of cDNA in the same PCR tube. The internal standards were added to the PCR master buffer to minimize variability from pipetting errors. The amount of internal standard used in each assay was determined by preliminary dilution experiments to ensure that the ratio of cDNA to internal standard was close to unity when a typical sample (i.e., 1 µl of RT solution) was amplified. The number of PCR cycles and the annealing temperatures varied according to primer melting temperature and cDNA abundance. The PCR products were quantified by soaking the gel in SYBRgreen and determining fluorescence intensity with a FluorImager (Molecular Dynamics). Once optimal conditions were established for each assay, a standard curve was done with a fixed amount of internal standard and a wide range of cDNA (from 4–5 times less to 4–5 times more than the amount expected in a typical sample). In all cases, the ratio of cDNA to internal standard after PCR amplification was proportional to the preamplification ratio. Figure 2 shows two of these curves. All samples from both younger and older subjects were then examined within the same assay. All samples had ratios of cDNA to internal standard that were within the range of the standard curves.

The reagent used to extract RNA separates most of the DNA from the RNA. When sham RT reactions were done with RNA pooled from each age group (transcriptase omitted), PCR generated no bands corresponding to the cDNAs of any of the nuclear genes (for low-abundance transcripts, it was necessary to use primers corresponding to different exons). Minor contamination of the RNA with mtDNA was detected, presumably because of the high copy number or small size of mtDNA (this problem is not relevant to SAGE, in which there is selection of polyadenylated RNA). Because
there are no introns in mtDNA, the problem could not be avoided by choosing primers from different exons. The results from the sham RT reactions on pooled RNA from both age groups suggested that the amount of mtDNA contamination was not enough to influence the results significantly. To verify this conclusion, we analyzed the mtDNA tran-
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Data analysis. The statistical significance of differences in
the number of times specific SAGE tags were detected in each
sample was determined as recommended (1). RT-PCR results
are expressed as a percentage of the mean value in young
muscle, and differences between age groups were determined
by t-tests for independent samples. Means are presented
with SEs.
muscle (Table 1). The age-related reduction in transcripts encoding these enzymes may be secondary to the shift in fiber-type composition (reduced ratio of type 2 to type 1 in older muscle, as discussed above).

Many mRNAs encoding enzymes involved in mitochondrial electron transport and ATP synthesis were expressed at high levels and were less abundant in the sample from older muscle (Table 2). These include transcripts of both mtDNA and nuclear DNA. Two SAGE tags corresponding to mtDNA transcripts (cytochrome-c oxidase 3, cytochrome b) were detected more frequently in the sample from older muscle, but, as discussed below, these mRNAs were actually underexpressed in older muscle. Because so many mRNAs in this category were expressed at a lower level in older muscle, and because this effect cannot be explained by selective atrophy of type 2 fibers in older muscle, the electron transport and ATP synthase mRNAs were the focus of the RT-PCR assays.

**Quantitative RT-PCR.** Figure 3 shows the mean relative concentrations of several mRNAs according to RT-PCR of individual samples. In agreement with SAGE, there was no age-related difference in the concentrations of mRNAs encoding α-actin, myoglobin, or creatine kinase. The modest decline in type 1 MHC mRNA suggested by SAGE was not verified by RT-PCR, but the decline in type 2a MHC mRNA was confirmed. According to RT-PCR, the average 2a:1 ratio was 41% less in older muscle ($P = 0.03$). Type 2x MHC mRNA expression was highly variable, especially

### Table 1. SAGE tags corresponding to mRNAs encoding proteins involved in glucose or glycogen metabolism that were detected less frequently in cDNA from older muscle than in cDNA from young muscle

<table>
<thead>
<tr>
<th>Tag, CATG +...</th>
<th>Gene Product</th>
<th>No. of Times Detected in cDNA From Young Muscle</th>
<th>No. of Times Detected in cDNA From Older Muscle</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACCATCAAT</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>593</td>
<td>394</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AGGATCGAGG</td>
<td>β-Enolase</td>
<td>324</td>
<td>175</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAGAGGTTGG</td>
<td>Glycogen phosphorylase</td>
<td>285</td>
<td>207</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GAGGCTGTGG</td>
<td>Phosphoglycerate mutase</td>
<td>186</td>
<td>93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGAGGGAATA</td>
<td>Triosephosphate isomerase</td>
<td>303</td>
<td>46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGCCCCACC</td>
<td>Pyruvate kinase</td>
<td>80</td>
<td>44</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>TGTCGCTGTC</td>
<td>Glycogen synthase</td>
<td>30</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GAAAACAGT</td>
<td>Phosphoglycerate kinase</td>
<td>16</td>
<td>4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

SAGE, serial analysis of gene expression.

### Table 2. SAGE tags corresponding to mRNAs encoding electron transport/ATP synthase proteins that were detected less frequently in cDNA from older muscle than in cDNA from young muscle

<table>
<thead>
<tr>
<th>Tag, CATG +...</th>
<th>Gene Product</th>
<th>No. of Times Detected in cDNA From Young Muscle</th>
<th>No. of Times Detected in cDNA From Older Muscle</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCCCACGTCC</td>
<td>Cytochrome-c oxidase 2</td>
<td>1,780</td>
<td>1,238</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CACCTAATTG</td>
<td>ATP synthase 6/8</td>
<td>1,304</td>
<td>936</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AGGCCCTACA</td>
<td>NADH dehydrogenase 3</td>
<td>702</td>
<td>553</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACCCTGCGCC</td>
<td>NADH dehydrogenase 1</td>
<td>565</td>
<td>406†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGGAGGCCCC</td>
<td>NADH dehydrogenase 4L†</td>
<td>169</td>
<td>103</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCCATCCTCT</td>
<td>ATP synthase 8‡</td>
<td>88</td>
<td>48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGGCGCTGTC</td>
<td>Ubiquinol cytochrome c reductase small subunit</td>
<td>78</td>
<td>46</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>GTGACCTCT</td>
<td>Cytochrome c oxidase 8</td>
<td>76</td>
<td>48</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TTGGGCGCTT</td>
<td>ATP synthase α</td>
<td>76</td>
<td>48</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TAGTGAAGT</td>
<td>Ubiquinone binding protein</td>
<td>57</td>
<td>33</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>GGGGCGTCACC</td>
<td>ATP synthase 9 (P1 gene)</td>
<td>47</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CTTATTCTCT</td>
<td>Cytochrome-c oxidase 4</td>
<td>46</td>
<td>22</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>AAAATAAAG</td>
<td>ATP synthase α</td>
<td>45</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGAATGACG</td>
<td>ATP synthase 9 (P3 gene)</td>
<td>41</td>
<td>20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CATTTCATAA</td>
<td>ATP synthase coupling factor 6</td>
<td>36</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGGGATGCTG</td>
<td>NADH dehydrogenase 51-kDa subunit</td>
<td>38</td>
<td>22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CTTGGAAGCT</td>
<td>NADH dehydrogenase 24-kDa subunit</td>
<td>25</td>
<td>12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AAGTCATTCA</td>
<td>NADH dehydrogenase B14</td>
<td>21</td>
<td>10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TGAGTCCTAG</td>
<td>NADH dehydrogenase UFS3</td>
<td>19</td>
<td>7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>GAATTCGTTA</td>
<td>NADH dehydrogenase 15-kDa subunit</td>
<td>18</td>
<td>7</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

*Two of the 8 older subjects had a polymorphism (C for T substitution at base 3640 of mitochondrial DNA, GenBank accession number X93334). This created a SAGE tag corresponding to the 3’ end of the NADH dehydrogenase 1 mRNA that was detected 268 times in the older sample but was not present in the younger sample. †The NADH dehydrogenase 4 and 4L mRNAs are part of the same transcript that is alternately translated by frame shifting. This tag represents a truncated transcript that can produce NADH dehydrogenase 4L but not NADH dehydrogenase 4. ‡The ATP synthase 6 and 8 mRNAs are part of the same transcript that is alternately translated by frame shifting. This tag represents a truncated transcript that can produce ATP synthase 8 but not ATP synthase 6.
among the younger subjects, so that there was no statistically significant difference between age groups. The 50% reduction in older muscle of phosphoglycerate mutase mRNA that was suggested by SAGE was confirmed by RT-PCR. However, the mean decline in glycogen synthase mRNA in older muscle was only 18% (not significant) according to RT-PCR, less than the 70% decline suggested by SAGE.

Seven of the transcripts listed in Table 2 were examined by quantitative RT-PCR. In agreement with SAGE, these mRNAs were less abundant in older muscle. For four of these transcripts (cytochrome-c oxidase 2 and 4, ATP synthase 6/8, NADH dehydrogenase 3), SAGE and RT-PCR indicated similar magnitudes for the relative reductions in older muscle. For three transcripts (ATP synthase α, ATP synthase coupling factor 6, NADH dehydrogenase 15-kDa subunit), the magnitude of the age-related difference, while highly significant (P<0.001), was about one-half the magnitude suggested by SAGE. According to RT-PCR, the mean levels of mRNAs encoding proteins involved in electron transport and ATP synthesis were 26–36% less in older muscle.

SAGE suggested that two mtDNA transcripts, cytochrome-c oxidase 3 and cytochrome b mRNAs, were more abundant in older muscle than in younger muscle. This result was unexpected because the mRNAs encoded by mtDNA are spliced from a single primary transcript, and several other mRNAs encoded by mtDNA were less abundant in older muscle. RT-PCR indicated that these mRNAs were underexpressed in older muscle by about the same amount as the other mtDNA transcripts. This discrepancy between SAGE and RT-PCR pointed to a flaw in the SAGE inventory. Apparently there was a bias toward detecting a tag less frequently in the young sample when the tag corre-
sponds to a sequence >500 bases from the polyadenylation site (as is the case for cytochrome-c oxidase 3 and cytochrome b tags). When cDNA was prepared from pooled mRNA for the production of SAGE tags, the RT reactions were not done simultaneously for the younger and older samples. The reaction appeared to be less efficient at producing longer cDNAs when the mRNA pool from young muscle was reverse transcribed, resulting in underrepresentation of transcripts when the SAGE sequence is far upstream from the polyadenylation site. This hypothesis was confirmed by the fact that cDNA (an aliquot that was not digested with NlaIII) corresponding to the 5’ end of cytochrome b mRNA was less abundant in the pool from young muscle, whereas cDNA corresponding to the 3’ end of cytochrome b mRNA was more abundant. This problem pertains only to the cDNA that was used to make SAGE tags, because all cDNA samples used in the RT-PCR assays were prepared simultaneously.

In RNA samples that were treated with DNase, there was no significant age-related difference in the mRNAs encoding α-actin (P = 0.12), and type 1 MHC (P = 0.55), consistent with the results from the same RNA samples not treated with DNase. Also consistent with the data obtained from the same RNA before DNase treatment, the DNased RNA from older muscle had significantly lower (35–50%) levels of mtDNA transcripts encoding ATP synthase 6/8 (P = 0.004), cytochrome-c oxidase 2 (P = 0.002) and 3 (P = 0.001), NADH dehydrogenase 1 (P = 0.01) and 3 (P = 0.007), and cytochrome b (P = 0.003).

The mitochondrial RNA polymerase mRNAs concentrations were similar in younger and older muscle. There was a slight difference in mtTFA mRNA levels between young and old samples (Fig. 3) that achieved marginal statistical significance.

The interindividual variability within each age group, as reflected by SDs and ranges, was generally similar. There was no evidence that the age-related differences suggested by SAGE were the result of one or a few individuals with unusual patterns of gene expression.

DISCUSSION

The role, if any, of altered gene expression in the etiology or adaptation to reduced muscle mass and performance in old age is unclear. To begin to address this issue, we used SAGE to compare levels of mRNAs in younger and older muscle. SAGE is a powerful tool because it provides relative quantitation of thousands of different transcripts. Although precision is poor for tags detected infrequently (1), we detected several hundred tags frequently enough to be fairly confident of the mean expression level of the corresponding mRNA.

The SAGE database reflects the average gene expression pattern in muscle pooled from several individuals within each age group. It does not provide any information about individual variation. In the catalog from young muscle, there was underrepresentation of transcripts for when the SAGE tag is far from the 3’ end (see RESULTS for explanation). For these reasons, the SAGE results are tentative, and any of the suggested age-related differences should be confirmed with another method before being accepted. Despite the limitations, SAGE was useful in pointing to many differentially expressed transcripts. In most cases that we tested, the differences in mRNA levels between younger and older muscle that were suggested by SAGE were confirmed by quantitative RT-PCR.

In any comparison of mRNA concentrations between groups, the denominator is important. With SAGE, each mRNA species is quantified in relation to the total pool of all mRNAs. Because the concentration of polyadenylated RNA per milligram tissue was similar in younger and older muscle, the SAGE data also reflect age-related differences in mRNA concentrations per milligram tissue. With the RT-PCR method used in the present study, each mRNA was quantified in relation to total RNA, because the same amount of RNA was used in every RT reaction. Total RNA levels were similar in younger and older muscle, so that the results from the RT-PCR method also reflect mRNA per milligram tissue. Another issue in comparing young and old muscle is the cellular source of the RNA. Although most of the mass of muscle tissue is multinuclear muscle fibers, small mononuclear cells (e.g., fibroblasts, endothelial cells, satellite cells) account for ~25% of the nuclei in a muscle sample (26). This proportion is similar in young and old muscle (26); thus it is unlikely that older muscle has a higher proportion of RNA from mononuclear cells. The fact that levels of several muscle-specific transcripts (α-actin, myoglobin, creatine kinase) are similar in younger and older muscle also indicates that the age-related differences observed in the present study are not caused by a difference in the proportion of RNA from mononuclear cells.

Although the distribution of mRNA between muscle fibers and mononuclear cells is probably similar in young and old muscle, the contribution of type 1 and type 2 muscle fibers to the mRNA pool is affected by age. According to both SAGE and RT-PCR, the ratio of type 2a MHC mRNA to type 1 MHC mRNA is reduced in older muscle. These results are consistent with the general finding that type 2 muscle fibers tend to atrophy during aging more than type 1 fibers (17). Selective type 2 fiber atrophy in older muscle might explain, at least in part, the lower levels of mRNAs encoding enzymes involved in glucose and glycolgen metabolism. This hypothesis must be verified by in situ hybridization or some other method that allows mRNA levels in type 1 and type 2 fibers to be measured separately.

Because mitochondrial mass, respiratory enzyme activity, and mtDNA transcript levels are greater in type 1 fibers (21, 23), a higher ratio of type 1 to type 2 fiber mass in older muscle would be expected to result in increased levels of mtDNA transcripts and other mRNAs encoding mitochondrial proteins. However, both SAGE and quantitative RT-PCR indicated that concentrations of many mRNAs encoding proteins involved in mitochondrial electron transport and ATP synthesis are less in old muscle than in young adult muscle. These data suggest that
reduced gene transcription or mRNA stability in older muscle may contribute to the declines in NADH dehydrogenase activity, cytochrome-c oxidase activity, and ATP regenerating capacity, which have been described in older muscle (8, 9, 18, 22, 24). It is unclear whether there is a decline in older muscle in concentrations of the electron transport complexes (3, 5). Even if the concentrations of these proteins are not different in younger and older muscle, reduced mRNA concentrations could theoretically impair enzyme-specific activity by slowing protein turnover (22). Slow turnover prolongs exposure of proteins to attack from reactive oxygen species, which may be an especially significant problem in mitochondria because they generate reactive oxygen species.

A few of the mRNAs encoding electron transport and ATP synthase proteins are transcripts of mtDNA. Previous studies of various tissues of Drosophila and rats, including skeletal muscle, indicated that aging is associated with reduced levels of these mRNAs (2, 6, 12, 13). In rat brain, the decline in mtDNA transcripts with aging appears to result from reduced transcription rather than reduced mRNA stability (12). In theory, a reduction in mtDNA transcripts in older muscle could be mediated by a reduced number of copies of mtDNA. In rat brain and heart, and in Drosophila, the age-related decline in mtDNA transcripts occurs without a decline in the number of copies of mtDNA (6, 12, 13). However, a recent preliminary report indicated that in rat muscle the age-related decline in mtDNA transcripts was about the same as the decline in mtDNA content (2). In human fibroblasts from older donors, there is an accumulation of point mutations in the mtDNA control region for replication, which suggests a possible mechanism for reduced mtDNA copy number (19). The abundance of the mRNA encoding mitochondrial RNA polymerase, which is essential for transcription of mtDNA, was similar in younger and older muscle. There was only a slight (16%) reduction in older muscle of the mRNA encoding mtTFA, which enhances transcription of mtDNA (15, 20). Although it seems unlikely that transcription of the mitochondrial RNA polymerase and mtTFA genes is reduced significantly in older muscle, we cannot discount the possibility that the concentration or activity of these factors within the mitochondria is affected by aging.

The age-related decline in expression of genes encoding proteins involved in electron transport and ATP synthesis might reflect a more sedentary lifestyle in the older men. To minimize differences in recent antecedent exercise, we asked subjects to refrain from activities more strenuous than walking for 3 days before the muscle biopsy was taken. We also excluded subjects who regularly exercised more than 2 h/wk. However, these precautions certainly do not guarantee that differences in physical activity did not have some role in the age-related differences observed in this study. It is interesting to note that hindlimb unweighting, a rodent model of muscle disuse, increases expression of glyceraldehyde-3-phosphate dehydrogenase, creatine kinase, and MHC 2x mRNAs in muscle (10), whereas older human muscle had reduced glyceraldehyde-3-phosphate dehydrogenase mRNA and no increase in either creatine kinase or MHC 2x mRNAs.

The decline in older muscle of transcripts encoding proteins involved in energy metabolism is consistent with a recent study in which a gene array method was used to search for transcripts differentially expressed in muscle of young and old mice (16). Older mice had increased expression in muscle of some mRNAs encoding proteins involved in stress responses (heat shock proteins, proteins induced by DNA damage and oxidative stress) and recovery from neuronal injury (proteins involved in reinnervation and neurite sprouting). SAGE did not detect these mRNAs frequently enough to allow any conclusions regarding the effect of age in human muscle.

Many of the age-related differences suggested by SAGE involve transcripts for which the protein product, or even the full mRNA sequence, has not been characterized. These match longer sequence tags in the GenBank expressed sequence tag database. Some of these may be identified within the next few years in the course of the sequencing of the human genome. Characterization of the proteins produced by translation of these mRNAs might provide novel information about the etiology of, or adaptation to, impaired muscle function in old age.

Aside from the issue of age-related differences, the database of over 100,000 SAGE tags may be a useful resource for investigators interested in the relative expression levels of mRNAs in human muscle. Quantitation should be fairly accurate for the most abundant mRNA species. For low-abundance transcripts, the quantitative precision is less but sufficient to differentiate them from the transcripts expressed at high-to-moderate levels.

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