J. Biochem. 132, 309–315 (2002)

Effects of Aging and Denervation on the Expression of Uncoupling Proteins in Slow- and Fast-Twitch Muscles of Rats[1]

Yasuhide Kontani,*,† Zuocheng Wang,*,‡ Tatsuo Furuyama,* Yuzo Sato,‡ Nozomu Mori,* and Hitoshi Yamashita*,[2]

Department of Molecular Genetics, National Institute for Longevity Sciences, and †Japan Foundation for Aging and Health, 36-3 Gengo, Obu 474-8522; and ‡Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya 464-8601

Received April 2, 2002; accepted June 6, 2002

We investigated the effects of aging and denervation on the gene expression of uncoupling proteins (UCPs) in slow-twitch soleus and fast-twitch gastrocnemius muscles. In a comparison between the control limbs of 6- and 24-month-old rats, the mRNA levels of UCP3, heart-type fatty acid binding protein (HFABP), and glucose transporter–4 (GLUT4) were considerably lower in the gastrocnemius muscles of the older rats, whereas no significant differences in the mRNA levels of those genes as well as UCP2 and cytochrome oxidase subunit IV (COX-IV) were observed in the soleus muscles of young and old rats. The UCP3 and COX-IV protein levels were also reduced considerably in the aged gastrocnemius muscles with atrophy. Denervation of the sciatic nerve caused an increase in UCP3 mRNA levels in both muscles, but the regulation of other genes contrasted between the two types of skeletal muscles. In spite of the increased mRNA level, a remarkable reduction in UCP3 protein was found in the denervated gastrocnemius muscles. These results indicate that the effects of aging and denervation on the gene expression of UCPs, HFABP, GLUT4, and COX-IV are different between the muscle types. The reduction in the mitochondrial UCP3 and COX proteins in aged fast-twitch muscles may have a negative effect on energy metabolism and thermogenesis in old animals.

Key words: aging, atrophy, energy metabolism, skeletal muscles, UCP.

Mitochondrial uncoupling proteins (UCPs), which have the potential to dissipate caloric energy as heat by uncoupling oxidative phosphorylation, are thought to play important roles in energy metabolism and thermogenesis (*1*). UCP1 is expressed in brown fat and is crucial for tolerance to cold (*2*). UCP2 and UCP3 are expressed ubiquitously and preferentially in muscle and brown fat, respectively (*3, 4*). A number of studies have shown the roles of UCP2 in lipid metabolism (*5*), the generation of reactive oxygen species (*6*), and the regulation of insulin secretion (*7*). Likewise, a recent study using mice overexpressing UCP3 in skeletal muscles provided evidence for the roles of UCP3 in energy metabolism, especially in glucose homeostasis (*8*). The data from that study also indicated that one UCP3 function is connected intimately with thermogenesis, because the muscle temperature was higher in the UCP3 transgenic mice. In the mitochondria of skeletal muscles, the use of two major energy substrates other than phosphorylcreatine, glucose and fatty acids, differs between muscle fiber types (*9*) and depends on the dominant action of glucose transporter–4 (GLUT4) (*10*) or heart-type fatty acid binding protein (HFABP) (*11*) expressed in the muscle.

The atrophy of skeletal muscles with dysfunction in the aging process, referred to as sarcopenia, is a general phenomenon and seems to originate from a decline in neuronal control or malnutrition (*12*). The muscle dysfunction resluts not only in inactivity but also probably a decrease in energy expenditure and/or impaired cold tolerance, because skeletal muscle, the biggest organ in the body, plays crucial roles in energy metabolism and thermogenesis. The dissipation of the mitochondrial proton gradient in skeletal muscles has been reported to reach 50% of the resting metabolic rate (*13*). The capacity for thermogenesis also tends to be attenuated with age (*14*). The muscle dysfunction with aging may contribute to increased adiposity and decreased cold tolerance, although the intolerance to cold in aged animals may be explained in part by the decline in UCP1 thermogenic ability (*15*) and the alteration in the inducibility of UCP genes in brown fat with aging (*16*). Most recently, Barazzoni and Nair demonstrated that UCP2 and UCP3 expression in rat fast-twitch gastrocnemius muscle changes with age (*17*); however, the regulation of UCPs in the aging skeletal muscles remains to be understood.

Muscle denervation has been used as a model of muscle immobility and atrophy to study the molecular mechanism regulating muscle activity. Dramatic changes occur in the

[1] This work was supported by a Research Grant for Longevity Sciences (11C–04) and by Health Sciences Research Grants, Comprehensive Research on Aging and Health, from the Ministry of Health, Labor and Welfare (to H.Y.), and also, in part, by a CREST grant from the Science and Technology Corporation of Japan (to N.M.).

[2] To whom correspondence should be addressed. Tel: +81-562-44-5651, Fax: +81-562-46-3157, E-mail: hyamashi@nils.go.jp

Abbreviations: UCP, uncoupling protein; HFABP, heart-type fatty acid binding protein; GLUT4, glucose transporter–4; COX-IV, cytochrome oxidase subunit IV.

morphological and biochemical properties of skeletal muscles after dissection of the motor nerve (*18, 19*). Some of these changes are thought to be associated with a remarkable change in energy metabolism in the immobilized muscles. Thus, it is worthwhile to study the regulation and role of UCPs in muscle atrophy induced acutely by denervation as well as that induced chronically by aging. To date, two groups have reported changes in UCP2 and UCP3 gene expression in gastrocnemius muscles following denervation, but the results were opposite between mice and rats (*20, 21*). In addition, there is no information about the effects of aging and denervation on the regulation of UCP expression in slow-twitch oxidative muscles.

Here, we report the regulation of UCP2 and UCP3 expression, as well as the gene expression of GLUT4, HFABP and cytochrome oxidase subunit IV (COX-IV) in two kinds of skeletal muscle, each having a different fiber type, from aged and denervated rats.

MATERIALS AND METHODS

Animals—Male young (6-month-old) and old (24-month-old) F344/N rats were obtained from the aging farm of the National Institute for Longevity Sciences (NILS). Rats were kept in cages at 23°C under controlled conditions with a 12-h:12-h light-dark cycle and consumed a regular chow diet and tap water *ad libitum*. Animal care and all experiments were carried out according to the institutional guidelines of NILS. The animals were anesthetized with pentobarbital sodium, after which denervation of the left hindlimb was carried out through the sciatic nerve section as previously described (*18*). The contralateral limb was used as the control. We did not perform a sham operation on the contralateral limb, because there was no difference in mRNA and protein levels of the genes examined in the present study between rats undergoing and not undergoing the sham operation in a preliminary experiment. One week after surgery, the rats were killed by decapitation. Soleus (slow-twitch, oxidative) and white gastrocnemius (fast-twitch, glycolytic) muscles were dissected carefully and used immediately for the isolation of RNA or mitochondria. Some parts of the gastrocnemius muscles were stored in liquid nitrogen for later analysis of chemical components. Ariano *et al.* have reported that rat soleus and gastrocnemius muscles consist of about 84% slow-twitch and 95% fast-twitch fibers, respectively (*9*), although the percent distribution of the two fiber types in gastrocnemius muscles is similar in normal humans. The major fiber type of the gastrocnemius muscles used was confirmed to be fast-twitch by immunohistological analysis (data not shown).

Determination of Chemical Components—For the determination of triglyceride and free fatty acid contents, tissue blocks were placed in glass tubes, and the lipid components were extracted with 2:1 (v/v) chloroform:methanol according to the method of Marshall *et al.* (*22*). The organic phase was evaporated under negative pressure, leaving a clearly visible lipid film at the bottom of the tube. The film was resuspended carefully in the reaction mixture provided with the kits for the determination of triglyceride or free fatty acids (Wako, Japan). The determination of glycogen in skeletal muscles was performed as previously described (*23*).

Northern Blot Analysis—Total RNA (15 or 20 μg per lane), isolated from the soleus and gastorocnemius muscles using the Trizol reagent (GIBCO BRL, USA), was analyzed by Northern blotting as described previously (*2*). Blots were hybridized successively with probes (labeled with [α-^{32}P]-dCTP) for the mRNAs of UCP2 (*2*), UCP3 (*16*), HFABP, GLUT4, COX-IV, and 18S rRNA (*2*). The cDNA probes for HFABP, GLUT4, and COX-IV mRNAs were produced from positions 7 to 459 of the rat HFABP nucleotide sequence (GeneBank accession no. M18034), from positions 765 to 1304 of the rat GLUT4 sequence (GeneBank accession no. J04524) and from positions 17 to 501 of the rat COX-IV sequence (GeneBank accession no. X15029), respectively, by a reverse transcription PCR technique. The PCR products were sequenced after subcloning into the pCRII vector (TA Cloning KIT, Invitrogen, CA). Hybridization signals were quantified by a Fuji Bioimage Analyzer. 18S rRNA was used to normalize variability in RNA loading, because the mRNA levels of β-actin and glyceraldehyde-3-phospate dehydrogenase were decreased significantly by denervation (data not shown).

Immunodetection of UCP3 and COX-IV Proteins—To examine the changes in the protein levels of UCP3 and COX-IV, we analyzed the mitochondrial and cytosolic proteins recovered from the soleus and gastrocnemius muscles by the Western blot method (*24*). Mitochondria were isolated from the muscles as previously described (*25*). The protein concentration of the samples was determined by the method of Bradford (BIO-RAD, USA). Mitochondrial proteins (10 μg) were separated on 5–20% acrylamide gel gradients and transferred onto polyvinylidene difluoride membranes (Immobilon; MILLIPORE, USA). UCP3 and COX-IV proteins were detected with affinity-purified rabbit polyclonal antibodies specific for UCP3 (STRATAGENE, USA) and a monoclonal antibody specific for COX-IV (Molecular Probes, USA) using an ECL detection system (Amarsham Pharmacia Biotech, UK). A specific signal for UCP3 was detected in the skeletal muscles, but not in liver, fat tissue, or spleen (data not shown). The UCP3 and COX-IV signals were quantified by Image Gauge ver. 3.3 (FUJI PHOTO FILM, Tokyo).

Statistical Analysis—The statistical significance of the data was assessed by ANOVA (Bonferroni/Dunn, Statview 5.0).

RESULTS

Effects of Aging and Denervation on Muscle Components—As shown in Table I, the tissue mass of the control soleus muscles was greater in the old rats than in the young ones, whereas there was no difference in the mass of control white gastrocnemius muscles between the young and old rats. On the other hand, the muscle tissue/body mass ratios were significantly lower in the old rats than in the young rats (8.6 and 22.8% decreases in soleus and gastrocnemius muscles, respectively, in the old rats), suggesting a tendency for sarcopenia to occur, especially in the gastrocnemius muscles of the old rats. The sectioning of the sciatic nerve resulted in atrophy of the skeletal muscles as previously reported elsewhere (*19*). Seven days after, denervation, a marked decrease in tissue mass was observed in both soleus and gastrocnemius muscles of the old rats (72.7 and 84.7% of control limbs, respectively), as well as in the muscles of the young rats (75.7 and 78.8% of control limbs,

respectively). Protein contents in the mitochondria from the gastrocnemius muscles, but not from the soleus muscles, were decreased by denervation, suggesting decreases in mitochondrial function and oxidative capacity in fast-twitch muscles. There was no significant difference in the mitochondrial protein contents of either muscle between the young and old rats.

Several biochemical parameters in the gastrocnemius muscles were then measured to see whether metabolic changes occurred in the muscles atrophied by age and denervation. The level of non-esterified fatty acids in the control limbs was significantly higher in the old rats than in the young rats, whereas the level was not changed by denervation. There was no significant difference in triglyceride level between the control and denervated limbs, although the level in the control limbs tended to be higher in the old rats than in the young rats ($p = 0.1$). Glycogen levels in the muscles did not change with age; however, the level was

TABLE I. **Effects of denervation on the tissue mass and chemical components of rat skeletal muscles.**

	Young rats		Old rats	
	Control (R)	Denervation (L)	Control (R)	Denervation (L)
Body mass (g)	351 ± 9 (n = 10)		430 ± 7[a] (n = 9)	
Soleus muscle				
(mg)	118 ± 4 (n = 10)	89 ± 4[b] (n = 10)	132 ± 3[c] (n = 9)	96 ± 2[b] (n = 9)
(mg/kg body mass)	337 ± 8 (n = 10)	255 ± 10[b] (n = 10)	308 ± 8[c] (n = 9)	224 ± 6[b] (n = 9)
Mitochondrial protein				
(mg recovered)	0.38 ± 0.03 (n = 5)	0.32 ± 0.04 (n = 5)	0.37 ± 0.03 (n = 4)	0.30 ± 0.04 (n = 4)
(mg/g tissue)	3.16 ± 0.22 (n = 5)	3.80 ± 0.43 (n = 5)	2.79 ± 0.20 (n = 4)	3.11 ± 0.35 (n = 4)
Gastrocnemius muscle				
(g)	1.75 ± 0.05 (n = 10)	1.38 ± 0.03[b] (n = 10)	1.65 ± 0.04 (n = 9)	1.40 ± 0.03[b] (n = 9)
(g/kg body mass)	4.99 ± 0.06 (n = 10)	3.93 ± 0.06[b] (n = 10)	3.85 ± 0.10[b] (n = 9)	3.26 ± 0.06[b] (n = 9)
Mitochondrial protein				
(mg recovered)	1.16 ± 0.10 (n = 10)	0.68 ± 0.15[c] (n = 10)	0.93 ± 0.14 (n = 9)	0.66 ± 0.10 (n = 9)
(mg/g tissue)	0.68 ± 0.07 (n = 10)	0.49 ± 0.10 (n = 10)	0.56 ± 0.08 (n = 9)	0.48 ± 0.07 (n = 9)
Non-esterified fatty acid				
(μEq/g tissue)	0.84 ± 0.04 (n = 5)	0.87 ± 0.06 (n = 5)	1.35 ± 0.21[d] (n = 9)	1.16 ± 0.11 (n = 9)
Triglyceride				
(mg/g tissue)	6.36 ± 0.09 (n = 5)	8.00 ± 2.76 (n = 5)	11.74 ± 2.02 (n = 9)	9.51 ± 2.18 (n = 9)
Glycogen				
(mg/g tissue)	5.44 ± 0.18 (n = 5)	6.52 ± 0.28 (n = 5)	5.63 ± 0.33 (n = 9)	8.53 ± 0.89[e] (n = 9)

The left hindlimbs (L) of rats were denervated and the right limbs (R) served as controls. Values are expressed as means ± SE. [a]$p < 0.001$ *vs.* young rats, [b]$p < 0.001$ *vs.* young or old control, [c]$p < 0.01$ *vs.* young control, [d]$p < 0.05$ *vs.* young control, [e]$p < 0.01$ *vs.* old control.

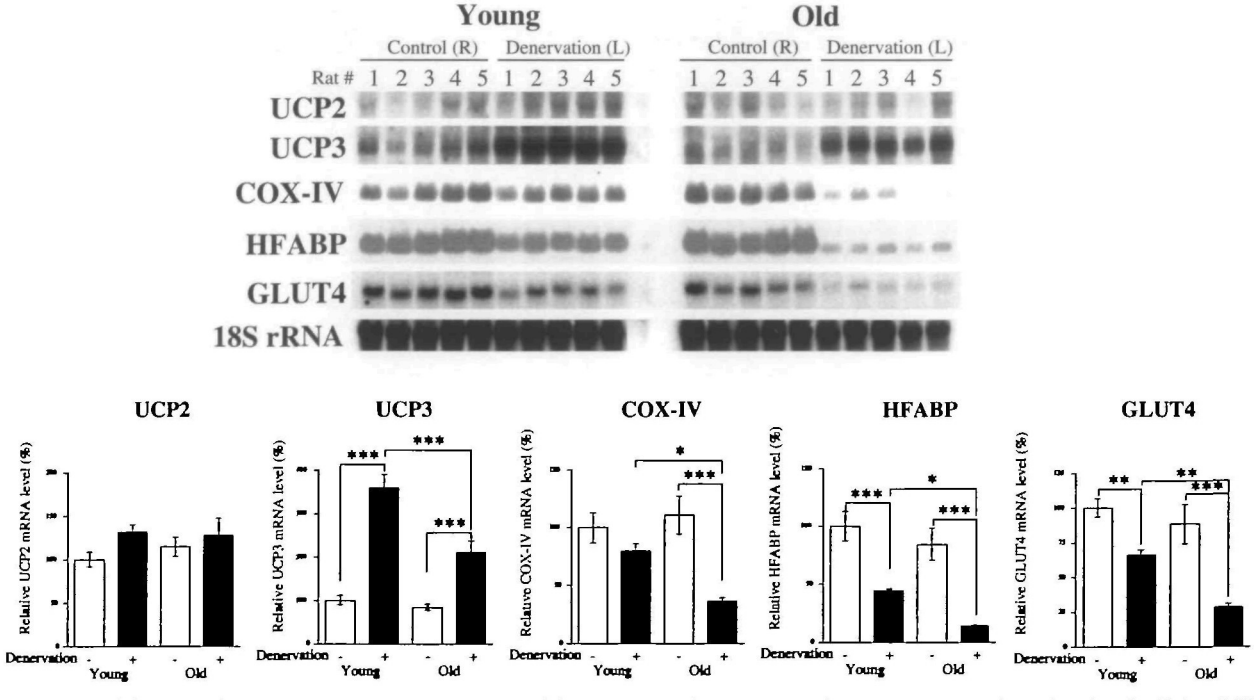

Fig. 1. **Effects of denervation on the expression of UCPs, COX-IV, HFABP, and GLUT4 genes in the soleus muscles of young and old rats.** Northern blot analyses using total RNA (15 μg) from soleus muscles were performed as described in "MATERIALS AND METHODS." The left hindlimb (L) was denervated and the right limb (R) served as a control. The relative mRNA levels of UCPs, COX-IV, HFABP, and GLUT4 are expressed as means ± SE (n = 5 for young and old rats). Statistical differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

increased significantly (1.5-fold) in the old rats by denervation.

Changes in Gene Expression in Skeletal Muscles with Aging and Denervation—Next we examined the mRNA levels of UCP2, UCP3, COX-IV, HFABP, and GLUT4. No significant differences in the levels of the mRNAs encoded by the 5 genes were observed in the soleus muscles of control limbs from the young and old rats (Fig. 1). In the control gastrocnemius muscles, however, the mRNA levels of UCP3, HFABP, and GLUT4 were considerably lower (47.5, 34.4, and 27.4%, respectively) in the old rats than in the young rats (Fig. 2). We could not detect any change in the UCP2 mRNA level in the muscles of the old rats, although Barazzoni and Nair recently reported increased UCP2 expression in gastrocnemius muscles from 27-month-old rats (*17*).

Denervation had a profound effect on gene expression in the skeletal muscles, but the effect was different between the young and old rats. In the soleus muscles (Fig. 1), the UCP3 mRNA level increased 3.6- and 2.5-fold by denervation in the young and old rats, respectively, although the UCP2 mRNA level did not change significantly in either group. In contrast, the HFABP mRNA level was decreased by denervation to 44.3 and 13.6% of the control level in the young and old rats, respectively. Likewise, the GLUT4 mRNA level was reduced to 65.7 and 28.3% of the control level in the young and old rats, respectively, by denervation. There was no significant effect of denervation on the COX-IV mRNA level in the young rats, whereas the mRNA level was reduced to 32.6% of the control level in the old rats. In gastrocnemius muscles (Fig. 2), denervation resulted in an increase in the UCP2 mRNA level of 3.0- and 2.3-fold in the young and old rats, respectively, and in the UCP3 mRNA level of 2.6- and 3.6-fold in the young and old rats, respectively. There was no effect of denervation on the gene expression of HFABP in the young rats, but the mRNA level in the old rats was increased 1.5-fold, which is the opposite of the response in aged soleus muscles. The GLUT4 mRNA level was reduced to 46.3 and 48.9% of the control level in the young and old rats, respectively, following denervation. There was no effect of denervation on the gene expression of COX-IV in the young and old rats. We also examined the gene expression in skeletal muscles 3 days following denervation, but the results were similar to those at 7 days (data not shown).

Regulation of UCP3 and COX-IV Protein Levels in the Mitochondria of Skeletal Muscles of Young and Old Rats After Denervation—We detected UCP3 and COX-IV proteins in mitochondria recovered from rat skeletal muscles. No signal for UCP3 was detected in the cytosolic fraction of the muscles (data not shown). There was no difference in the UCP3 protein level in the mitochondria of the control soleus muscles between the young and old rats (Fig. 3), whereas in the mitochondria of the control gastrocnemius muscles, the UCP3 protein level of the old rats was about half of that of the young rats (Fig. 4). Because the protein content (mg/g tissue) in mitochondria from the gastrocnemius muscles, but not in those from the soleus muscles,

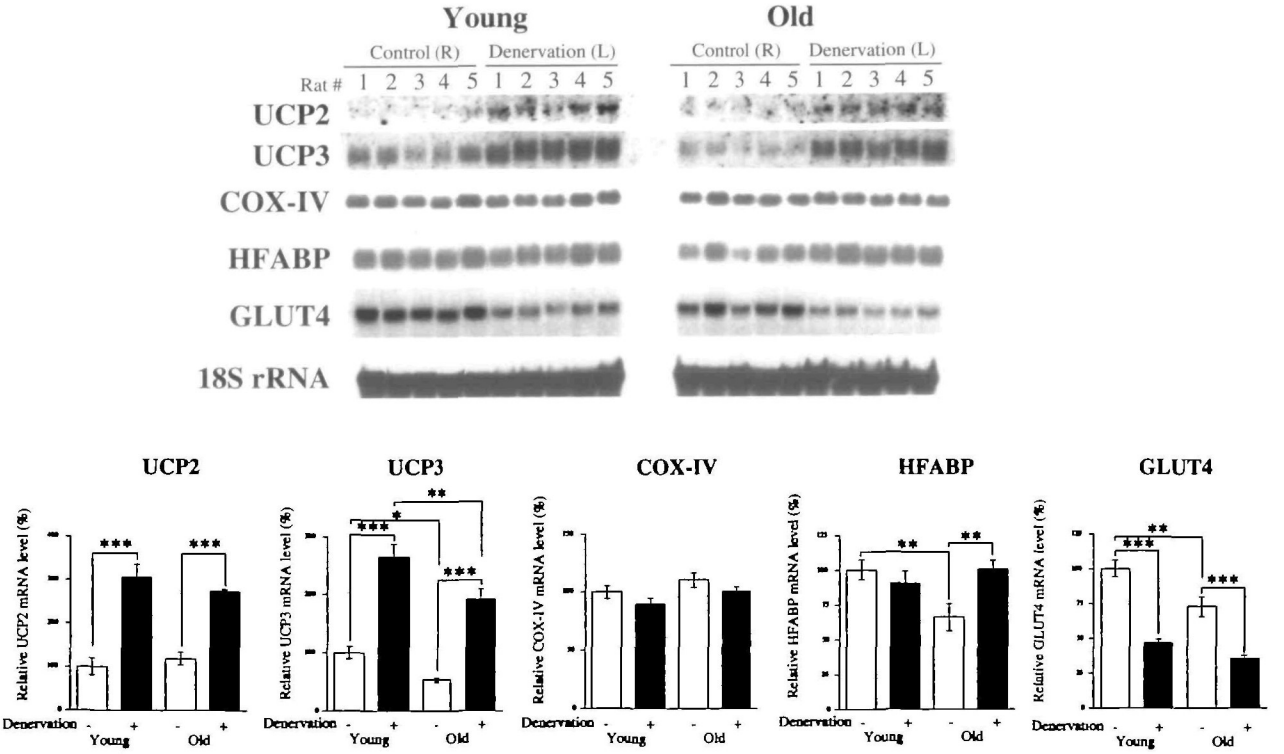

Fig. 2. **Effects of denervation on the expression of UCPs, COX-IV, HFABP, and GLUT4 genes in the gastrocnemius muscles of young and old rats.** Northern blots using total RNA (20 μg) from gastrocnemius muscles were performed as described in "MATERIALS AND METHODS." The left hindlimb (L) was denervated and the right limb (R) served as a control. The relative mRNA levels of UCPs, COX-IV, HFABP, and GLUT4 are expressed as means ± SE ($n = 5$ for young and old rats). Statistical differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

was lower in the denervated limbs than in the control limbs (Table I), the total UCP3 protein level in the denervated gastrocnemius muscles is considered to be much lower than that in the control. The COX-IV protein levels in the soleus and gastrocnemius muscles of the control limbs were significantly lower (25.3 and 44.9% decreases, respectively) in the old rats than in the young rats. As a result, the UCP3/COX-IV protein ratio of the old rats was higher in the soleus muscles and much lower in the gastrocnemius muscles compared with the ratios in the young rats (Figs. 3 and 4).

Denervation remarkably changed the protein levels of UCP3 and COX-IV in the mitochondria of skeletal muscles. In the soleus muscles (Fig. 3), the UCP3 protein level in the young rats was decreased 21% compared with the control level, whereas there was no effect on the level in the old rats. The COX-IV protein levels in the young and old rats were decreased 11.3 and 28.8%, respectively, compared with the control levels. The ratio of UCP3/COX-IV protein in the mitochondria from the soleus muscles of the young rats did not change substantially, but increased significantly in mitochondria from old rats (Fig. 3). In the gastrocnemius muscles (Fig. 4), the UCP3 protein levels in the young and old rats were decreased 63.2 and 34.9%, respectively, compared with the control levels. The COX-IV protein levels in the young and old rats were also decreased,

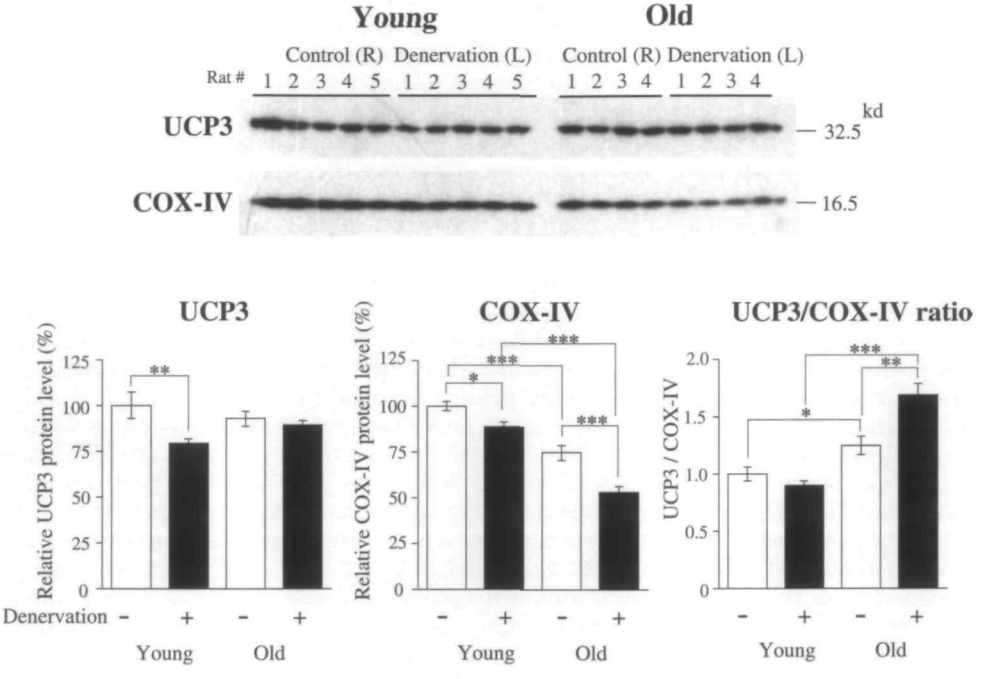

Fig. 3. **Western blot analysis for UCP3 and COX-IV in soleus muscles.** Mitochondrial proteins (10 μg) isolated from the soleus muscles of young and old rats were analyzed as described in "MATERIALS AND METHODS." The left hindlimb (L) of rats was denervated and the right limb (R) served as a control. Relative protein levels of UCP3 and COX-IV, and the ratio of UCP3/COX-IV proteins are expressed as means ± SE (young rats: $n = 5$; old rats: $n = 4$). Statistical differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

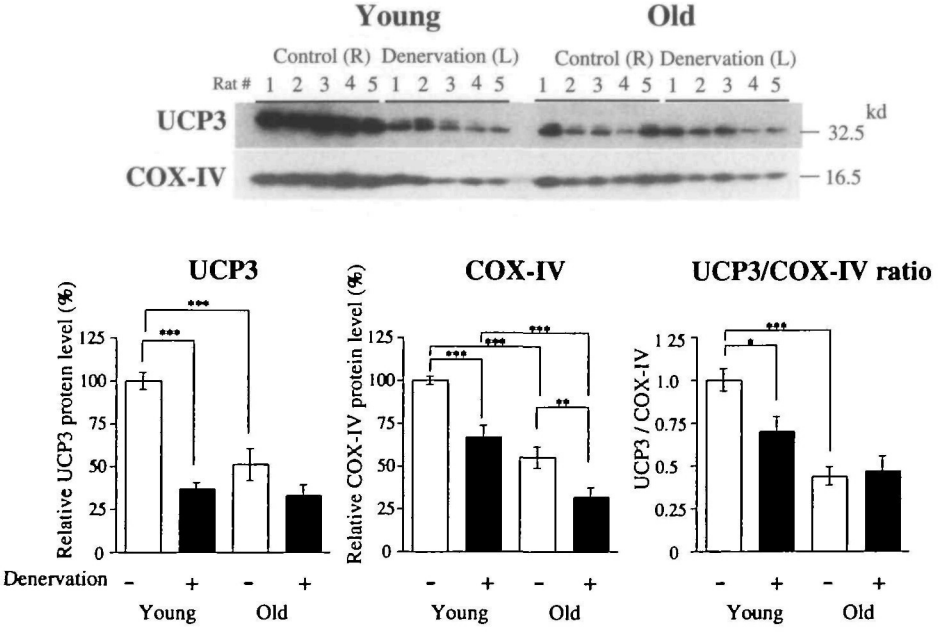

Fig. 4. **Western blot analysis for UCP3 and COX-IV in gastrocnemius muscles.** Mitochondrial proteins (10 μg) isolated from the gastrocnemius muscles of young and old rats were analyzed as described in "MATERIALS AND METHODS." The left hindlimb (L) of rats was denervated and the right limb (R) served as a control. Relative protein levels of UCP3 and COX-IV, and the ratio of UCP3/COX-IV proteins are expressed as means ± SE (young rats: $n = 10$; old rats: $n = 9$). Statistical differences are show as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

33.0 and 42.6%, respectively, compared with their control levels. The ratio of UCP3/COX-IV protein in mitochondria obtained from the denervated gastrocnemius muscles was significantly decreased in the case of the young rats, but did not change in the case of the old rats.

DISCUSSION

Skeletal muscle is one of most important organs involved in energy homeostasis and thermogenesis, the functions of which are known to be attenuated with age (*12*) or to be impaired by the bisection of motor neurons innervating the muscles (*26*). Likewise, muscle atrophy is induced chronically (sarcopenia) or acutely with aging or denervation, respectively. In this context, we studied the effects of aging and denervation on the gene expression of UCPs, COX-IV, HFABP, and GLUT4 in skeletal muscles, because these proteins are considered to act essentially in energy metabolism and in thermogenesis (*1, 5, 8, 10, 11*). It was of particular importance in the present study to examine whether those genes are regulated differentially in two different muscle types, *i.e.*, slow-twitch oxidative and fast-twitch glycolytic muscles. In the present study, the mRNA levels of UCP3, HFABP, and GLUT4 decreased considerably in the gastrocnemius muscles with age, whereas no significant difference in the mRNA levels of these 5 genes was observed in the soleus muscles of the control limbs between young and old rats, suggesting the attenuated translation and/or stability of genes involved in energy metabolism in aged fast-twitch muscles. The decreases in several genes in the aged gastrocnemius muscles are in agreement with the observations of the gene expression profile by Lee *et al.* (*27*) that aging results in a differential gene expression pattern indicative of a marked stress response and lower expression of metabolic genes in mouse gastrocnemius muscles. Similar patterns at the protein level were confirmed for the UCP3 protein in mitochondria recovered from the soleus and gastrocnemius muscles of the control limbs, although COX-IV protein levels decreased in both muscle types with age. Our results on the age-related change in UCP3 expression in gastrocnemius muscles are similar to those of Barazzoni and Nair (*17*), although we could not detect a significant increase in the UCP2 mRNA level in aging fast-twitch muscles. The three-month difference in rat age used between our study (24-month-old) and their study (27-month-old) might affect the results, because the mortality rate of rats increases markedly from about 50 to 75% during this period (*28*), suggesting a decline in the vital condition of rats. Shivering thermogenesis in skeletal muscles is an important part of homeothermy. In view of the dominant expression of UCP3 in skeletal muscles and the increase in muscle temperature in UCP3 transgenic mice (*8*), it is likely that the decrease in UCP3 expression in the fast-twitch muscles with aging is associated with the decline in cold tolerance seen in old animals, although the lack of brown fat non-shivering (UCP1) thermogenesis may be the principle factor in the mechanism (*15, 29*). COX plays a pivotal role in energy transfer in complex IV of the respiratory chain, and its activity is thought to reflect mitochondrial oxidative ability (*30*). In our study the decrease in COX-IV protein level in the mitochondria of skeletal muscles from old rats was also greater in the gastrocnemius muscles than in the soleus muscles. Recently, Huppertz *et al.* demonstrated that UCP3 stimulates glucose transport and GLUT4 translocation to the skeletal muscle cell surface by activating a phosphoinositide 3-kinase–dependent pathway (*31*). In addition, since mice that overexpress UCP3 in their skeletal muscles have an increased glucose clearance rate and strikingly reduced fat deposition (*8*), it is conceivable that the reduced expression of UCP3 and GLUT4 in the fast-twitch glycolytic muscles with age could cause impaired glucose tolerance and then lipid accumulation. In fact, the contents of non-esterified fatty acids and triglycerides in the gastrocnemius muscles tended to increase in the old heavier animals; however, a contribution of the decreased HFABP expression to the increase in lipid content can not be ruled out, because HFABP is crucial for fatty acid utilization by transporting them to mitochondria from the plasma membrane (*32*).

Recently the effect of denervation on the levels of the UCP2 and UCP3 mRNAs in the gastrocnemius muscles of mice and rats was reported (*20, 21*). Our results for gastrocnemius muscles are consistent with those of the rat experiments (*21*). We also found that denervation has profound effects on gene expression, chemical components, and mitochondrial oxidative capacity in skeletal muscles, but the effects differ in their fashion and magnitude between slow- and fast-twitch muscles and between young and old rats. Especially, the regulation of UCP2, COX-IV, and HFABP gene expression in the two muscle types contrasts and the downregulation of the genes examined was greater in the aged soleus muscles. These results suggest a functional difference in energy metabolism between fast- and slow-twitch muscles under denervated conditions. Nevertheless, the effect of denervation on the decreases in the UCP3 and COX-IV protein levels was greater in the gastrocnemius muscles than in the soleus muscles. We can not explain the discrepancy between the mRNA and protein levels in the denervated muscles, but it may result from changes in transcriptional regulation and/or the protein degradation system (Ub-proteasome/lysosome) in the atrophied muscles. There also might be a contribution of other cell types, such as interstitial cells, in the change in gene expression in the atrophied muscles. If the change in the UCP3/COX-IV ratio in skeletal muscles indicates an imbalance of energy dispensation, the decrease in the UCP3/COX-IV ratio seen in the denervated fast-twitch muscles of the young rats might be a defensive response to maintain ATP levels for tissue survival. On the other hand, the increase in this ratio in the denervated slow-twitch muscles of the old rats could be a compensatory response to the attenuated thermogenic ability of the fast-twitch muscles. The results also suggest an increased dependency on the slow-twitch oxidative muscles under inactive conditions. The high susceptibility of fast-twitch glycolytic muscles to an inactive condition seems to be reasonable, because the animals can not move their denervated legs quickly and powerfully, meaning disuse of the fast-twitch muscles. The slow-twitch muscles are probably more important for guaranteeing the minimum use of the crippled legs.

Thus, our data indicate that the transcriptional or translational regulation of UCP2, UCP3, HFABP, GLUT4, and COX-IV in the process of aging or muscle atrophy by denervation is quite different between the fast-twitch glycolytic muscles and the slow-twitch oxidative muscles. The present data also indicate that the age-dependent decline in the

functions of energy metabolism and thermogenesis is more severe in fast-twitch glycolytic muscles than in slow-twitch oxidative muscles. In particular, the notable reduction in the level of the UCP3 protein in the atrophied fast-twitch muscles with age strongly suggests decreased metabolic capacity and thermogenic ability, which may lead to the accumulation of excess energy as fat and to intolerance to cold in aged animals.

We are grateful to T. Mizuno, T. Suzuki and I. Nakano for technical assistance and laboratory maintenance.

REFERENCES

1. Ricquier, D. and Bouillaud, F. (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem. J.* **345**, 161–179
2. Enerback, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M.E., and Kozak, L.P. (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* **387**, 90–94
3. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D., and Warden, C.H. (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15**, 269–272
4. Mao, W., Yu, X.X., Zhong, A., Li, W., Brush, J., Sherwood, S.W., Adams, S.H., and Pan, G. (1999) UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. *FEBS Lett.* **443**, 326–330
5. Samec, S., Seydoux, J., and Dulloo, A.G. (1998) Role of UCP homologues in skeletal muscles and brown adipose tissue: mediators of thermogenesis or regulators of lipids as fuel substrate? *FASEB J.* **12**, 715–724
6. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B.S., Miroux, B., Couplan, E., Alves-Guerra, M.C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet.* **26**, 435–439
7. Zhang, C.Y., Baffy, G., Perret, P., Krauss, S., Peroni, O., Grujic, D., Hagen, T., Vidal-Puig, A.J., Boss, O., Kim, Y.B., Zheng, X.X., Wheeler, M.B., Shulman, G.I., Chan, C.B., and Lowell, B.B. (2001) Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* **105**, 745–755
8. Clapham, J.C., Arch, J.R., Chapman, H., Haynes, A., Lister, C., Moore, G.B., Piercy, V., Carter, S.A., Lehner, I., Smith, S.A., Beeley, L.J., Godden, R.J., Herrity, N., Skehel, M., Changani, K.K., Hockings, P.D., Reid, D.G., Squires, S.M., Hatcher, J., Trail, B., Latcham, J., Rastan, S., Harper, A.J., Cadenas, S., Buckingham, J.A., Brand, M.D., and Abuin, A. (2000) Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* **406**, 415–418
9. Ariano, M.A., Armstrong, R.B., and Edgerton, V.R. (1973) Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* **21**, 51–55
10. Marette, A., Richardson, J.M., Ramlal, T., Balon, T.W., Vranic, M., Pessin, J.E., and Klip, A. (1992) Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *Am. J. Physiol.* **263**, C443–452
11. Claffey, K.P., Herrera, V.L., Brecher, P., and Ruiz-Opazo, N. (1987) Cloning and tissue distribution of rat heart fatty acid binding protein mRNA: identical forms in heart and skeletal muscle. *Biochemistry* **26**, 7900–7904
12. Navarro, A., Lopez-Cepero, J.M., and Sanchez del Pino, M.J. (2001) Skeletal muscle and aging. *Front. Biosci.* **6**, D26–44
13. Rolfe, D.F. and Brand, M.D. (1996) Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. *Am. J. Physiol.* **271**, C1380–1389
14. Florez-Duquet, M. and McDonald, R.B. (1998) Cold-induced thermoregulation and biological aging. *Physiol. Rev.* **78**, 339–358
15. Yamashita, H., Yamamoto, M., Ookawara, T., Sato, Y., Ueno, N., and Ohno, H. (1994) Discordance between thermogenic activity and expression of uncoupling protein in brown adipose tissue of old rats. *J. Gerontol.* **49**, B54–59
16. Yamashita, H., Sato, Y., and Mori, N. (1999) Difference in induction of uncoupling protein genes in adipose tissues between young and old rats during cold exposure. *FEBS Lett.* **458**, 157–161
17. Barazzoni, R. and Nair, K.S. (2001) Changes in uncoupling protein-2 and -3 expression in aging rat skeletal muscle, liver, and heart. *Am. J. Physiol. Endocrinol. Metab.* **280**, E413–419
18. Coderre, L., Monfar, M.M., Chen, K.S., Heydrick, S.J., Kurowski, T.G., Ruderman, N.B., and Pilch, P.F. (1992) Alteration in the expression of GLUT-1 and GLUT-4 protein and messenger RNA levels in denervated rat muscles. *Endocrinology* **131**, 1821–1825
19. Furuno, K., Goodman, M.N., and Goldberg, A.L. (1990) Role of different proteolytic systems in the degradation of muscle proteins during denervation atrophy. *J. Biol. Chem.* **265**, 8550–8557
20. Tsuboyama-Kasaoka, N., Tsunoda, N., Maruyama, K., Takahashi, M., Kim, H., Ikemoto, S., and Ezaki, O. (1998) Up-regulation of uncoupling protein 3 (UCP3) mRNA by exercise training and down-regulation of UCP3 by denervation in skeletal muscles. *Biochem. Biophys. Res. Commun.* **247**, 498–503
21. Cortright, R.N., Zheng, D., Jones, J.P., Fluckey, J.D., DiCarlo, S.E., Grujic, D., Lowell, B.B., and Dohm, G.L. (1999) Regulation of skeletal muscle UCP-2 and UCP-3 gene expression by exercise and denervation. *Am. J. Physiol.* **276**, E217–221
22. Marshall, B.A., Tordjman, K., Host, H.H., Ensor, N.J., Kwon, G., Marshall, C.A., Coleman, T., McDaniel, M.L., and Semenkovich, C.F. (1999) Relative hypoglycemia and hyperinsulinemia in mice with heterozygous lipoprotein lipase (LPL) deficiency. Islet LPL regulates insulin secretion. *J. Biol. Chem.* **274**, 27426–27432
23. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956) *Anal. Chem.* **28**, 350–356
24. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354
25. Vidal-Puig, A.J., Grujic, D., Zhang, C.Y., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., Muoio, D.M., and Lowell, B.B. (2000) Energy metabolism in uncoupling protein 3 gene knockout mice. *J. Biol. Chem.* **275**, 16258–16266
26. Uehara, Y., Campbell, G.R., and Burnstock, G. (1976) Muscle and its innervation in *An Atlas of Fine Structure*, pp. 371–372, Edward Arnold, London
27. Lee, C-K, Klopp, R.G., Weindruch, R., and Prolla, T.A. (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* **285**, 1390–1393
28. Tanaka, S., Segawa, T., Tamaya, N., and Ohno, T. (2000) Establishment of an aging farm of F344/N rats and C57BL/6 mice at the National Institute for Longevity Sciences (NILS). *Arch. Gerontol. Geriatr.* **30**, 215–223
29. Golozoubova, V., Hohtola, E., Matthias, A., Jacobsson, A., Cannon, B., and Nedergaard, J. (2001) Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J.* **15**, 2048–2050
30. Newsholme, E.A. and Start, C. (1973) *Regulation in Metabolism*, John Wiley & Sons, New York
31. Huppertz, C., Fischer, B.M., Kim, Y.B., Kotani, K., Vidal-Puig, A., Slieker, L.J., Sloop, K.W., Lowell, B.B., and Kahn, B.B. (2001) Uncoupling protein 3 (UCP3) stimulates glucose uptake in muscle cells through a phosphoinositide 3-kinase-dependent mechanism. *J. Biol. Chem.* **276**, 12520–12529
32. Binas, B., Danneberg, H., McWhir, J., Mullins, L., and Clark, A.J. (1999) Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *FASEB J.* **13**, 805–812