Selenoprotein-Deficient Transgenic Mice Exhibit Enhanced Exercise-Induced Muscle Growth


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ABSTRACT Dietary intake of selenium has been implicated in a wide range of health issues, including aging, heart disease and cancer. Selenium deficiency, which can reduce selenoprotein levels, has been associated with several striated muscle pathologies. To investigate the role of selenoproteins in skeletal muscle biology, we used a transgenic mouse (referred to as i6A) that has reduced levels of selenoproteins due to the introduction and expression of a dominantly acting mutant form of selenocysteine transfer RNA (tRNA[Sec]). As a consequence, each organ contains reduced levels of most selenoproteins, yet these mice are normal with regard to fertility, overall health, behavior and blood chemistries. In the present study, although skeletal muscles from i6A mice were phenotypically indistinguishable from those of wild-type mice, plantaris muscles were ~50% heavier after synergist ablation, a model of exercise overload. Like muscle in wild-type mice, the enhanced growth in the i6A mice was completely blocked by inhibition of the mammalian target of rapamycin (mTOR) pathway. Muscles of transgenic mice exhibited increased site-specific phosphorylation on both Akt and p70 ribosomal S6 kinase (p70S6k) (P < 0.05) before ablation, perhaps accounting for the enhanced response to synergist ablation. Thus, a single genetic alteration resulted in enhanced skeletal muscle adaptation after exercise, and this is likely through subtle changes in the resting phosphorylation state of growth-related kinases. J. Nutr. 133: 3091–3097, 2003.

KEY WORDS: selenium • Akt/PKB • transgenic • signaling • p70 ribosomal S6 kinase

Dietary intake of selenium has been implicated in a broad range of human physiologic conditions and diseases, including aging, heart disease and cancer (1). Selenium deficiency, which can reduce selenoprotein levels, has been associated with several striated muscle pathologies. To investigate the role of selenoproteins in skeletal muscle biology, we used a transgenic mouse (referred to as i6A) that has reduced levels of selenoproteins due to the introduction and expression of a dominantly acting mutant form of selenocysteine transfer RNA (tRNA[Sec]). As a consequence, each organ contains reduced levels of most selenoproteins, yet these mice are normal with regard to fertility, overall health, behavior and blood chemistries. In the present study, although skeletal muscles from i6A mice were phenotypically indistinguishable from those of wild-type mice, plantaris muscles were ~50% heavier after synergist ablation, a model of exercise overload. Like muscle in wild-type mice, the enhanced growth in the i6A mice was completely blocked by inhibition of the mammalian target of rapamycin (mTOR) pathway. Muscles of transgenic mice exhibited increased site-specific phosphorylation on both Akt and p70 ribosomal S6 kinase (p70S6k) (P < 0.05) before ablation, perhaps accounting for the enhanced response to synergist ablation. Thus, a single genetic alteration resulted in enhanced skeletal muscle adaptation after exercise, and this is likely through subtle changes in the resting phosphorylation state of growth-related kinases. J. Nutr. 133: 3091–3097, 2003.

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3 Abbreviations used: ERK, extracellular regulated kinase; GPx-1, glutathione peroxidase 1; IPA, selenoprotein deficient; mTOR, mammalian target of rapamycin; PKB, protein kinase B; p70S6k, p70 ribosomal S6 kinase; SA, synergist ablation; SA, synergist ablation group; −SA, nonsynergist ablation group; Sec, selenocysteine; SeIP, selenoprotein P; SEPN1, selenoprotein N 1; TR1, thioreductase 1; tRNA[Sec], selenocysteine transfer RNA; WT, wild-type.
signaling molecules was shown to be affected by the addition of pharmacologically effective selenium-containing compounds, some of which are precursors of selenoproteins (12–15). However, a role for selenium in the regulation of skeletal muscle adaptation associated with increased mechanical loading has yet to be investigated.

Transgenic mice that overexpress a dominantly acting mutant form of tRNA[^Ser]Sec were described recently (16). These mice, in which a mutant tRNA is overexpressed in all tissues, have reduced levels of selenoproteins, such as glutathione peroxidase 1 (GPx-1), Thioreductase 1 (TR1) and selenoprotein P (SelP) in all tissues studied including the brain, liver and kidney (16). Although levels of several selenoproteins were reduced in the tissues studied, there was no apparent phenotype with these mice. This transgenic animal provides a unique model with which to examine the biological effects of reduced selenoprotein levels independently of other consequences that might occur in mice fed a selenium-deficient diet (16). In this study, these transgenic mice were subjected to a well-defined model of exercise, synergist ablation (SA) (11,17).

**MATERIALS AND METHODS**

**Animals and surgical procedures.** A generation of transgenic mice expressing a selenocysteine tRNA incapable of being modified to include i6A was described previously (16). Bilateral SA surgery was performed on 10- to 12-wk-old mice after anesthetization with sodium pentobarbital (40 mg/kg) as previously described (18,19).

**FIGURE 1** Relative plantaris muscle mass in wild-type (WT) and transgenic (i6A–) mice that did (+SA) or did not (−SA) undergo synergist ablation. Values are means ± SEM, n = 12–13 and are the percentage of WT/−SA. a Different from WT/−SA (P < 0.05). b Different from WT/+SA (P < 0.05).

**FIGURE 2** Cross-sections stained with hematoxylin and eosin of the control (−SA; panels A and B) and synergist ablated (+SA; panels C and D) plantaris muscles from wild-type (WT; panels A and C) and transgenic (i6A–; panels B and D) mice.

| TABLE 1 |

| Absolute plantaris muscle mass in wild-type (WT) and transgenic (i6A–) mice that did (+SA) or did not (−SA) undergo synergist ablation1 |
|---|---|
| mg |
| 12.7 ± 0.8bc | 36.2 ± 1.8bc |
| 13.9 ± 0.8bc | 53.8 ± 3.4ab |

1 Values are means ± SEM, n = 12–13. a Different from WT/−SA (P < 0.05); b Different from WT/+SA (P < 0.05); c different from i6A–/+SA (P < 0.05).
Briefly, this surgery involves removal of the gastrocnemius and soleus muscles leaving the plantaris muscle intact. Fourteen days after SA, the plantaris muscle was collected, quickly weighed and then frozen in liquid nitrogen. Mice were killed by injection with saturated KCl. These studies were conducted under a protocol approved by the Animal Care Committee at the University of Illinois at Chicago.

**Western blots.** Muscles where homogenized in a buffer containing 20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 10 g/L Nonidet NP40, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerolphosphate, 1 mmol/L sodium orthovanadate, 1 mg/L leupeptin and 1 mmol/L phenylmethylsulfonyl fluoride. The homogenate was clarified by centrifugation at 15,000 × g for 10 min and protein concentration of the supernatant was determined by the DC protein assay (Bio-Rad Laboratories, Hercules, CA). SDS-PAGE was performed on 7.5% acrylamide gels and proteins transferred to polyvinylidene difluoride or nitrocellulose membranes. Western blots were visualized with enhanced chemiluminescence Western blotting kit (Amersham Pharmacia Biotech, Piscataway, NJ). Antibodies for the Sel R and Sel T proteins were generously provided by Dr. Vadim Gladyshev (University of Nebraska, Lincoln, NE). Antibodies against p70S6k (Santa Cruz Biotechnology, Santa Cruz, CA), Akt, p38 and extracellular regulated kinase (ERK) (New England Biolabs, Beverly, MA) were used to determine the relative concentration of each protein. Phosphospecific antibodies against p70S6k 421/424, p70S6k 389, Akt 473, Akt 308, p38 180/182 and ERK 202/204 (New England Biolabs, Beverly, MA) were used to determine the relative concentration of each kinase in its phosphorylated/activated state. Using the general p70S6k antibody (Santa Cruz) resolves multiple bands after electrophoretic separation, with the slower migrating bands representing states of increased phosphorylation. The percentage of phosphorylation was quantified as previously described (8). Densitometric measurements were carried out on a FluorS Max Imager using QuantityOne Software (Bio-Rad Laboratories).

**Muscle histology.** Plantaris muscles from wild-type (WT) and selenoprotein deficient (i6A−) mice were dissected 14 d after SA, coated with tissue freezing medium (Fisher Scientific, Hanover Park, IL), frozen in melting isopentane cooled on dry ice and stored at −80°C. Cross sections were cut at 10 μm from the muscle midbelly using a cryostat and stained with hematoxylin and eosin. Briefly, muscles were mounted on Superfrost/Plus slides (Fisher Scientific), fixed in absolute methanol for 5 min and stained with hematoxylin and eosin. Muscle sections were viewed with a light microscope (Nikon Instruments, Melville, NY) and fibers examined for evidence of overt morphological abnormalities (400X), including in inflammatory cells, pale or diffuse staining cytoplasm, centrally located nuclei, small angular fibers and/or marked swollen appearance as previously described (20).

**Enzyme assays.** GPx-1 and TR1 activities were assayed as described in Moustafa et al. (21).

**Rapamycin experiments.** To assess the contribution of the mTOR signaling pathway to the enhanced muscle growth observed in the i6A− mice, rapamycin (Calbiochem, San Diego, CA: 1.5 mg/kg intraperitoneally) or vehicle (20 g/L carboxymethylcellulose, 2.5g/L Tween-20 intraperitoneally) was administered daily for 14 d in both control (−SA) and synergist ablated (+SA) i6A− mice. The groups consisted of: −Rap/−SA (n = 12 muscles), +Rap/−SA (n = 6 muscles), −Rap/+SA (n = 12 muscles), +Rap/+SA (n = 9 muscles).

**Statistical Analysis.** Statistical analysis for these studies was performed using t test or two-way ANOVA followed by Tukey's post-hoc analysis. Differences between groups were considered significant if P < 0.05.

**RESULTS AND DISCUSSION**

Previous studies indicated that numerous tissues in i6A− mice have reduced levels of selenoproteins, yet they appear phenotypically normal with regard to fertility, overall health, behavior and blood chemistries (16). For example, activity levels of GPx-1 were reduced ~60% in liver, kidney and brain, whereas levels of deiodinase 1 and TR1 were reduced ~60–70% in liver. Consistent with results in liver, GPx-1 activity in

![FIGURE 3 Changes in Akt phosphorylation and content in muscles of wild-type (WT) and transgenic (i6A−) mice that did (+SA) or did not (−SA) undergo synergist ablation. (A) Representative Western blots of phospho- and total Akt for all 4 groups. Quantification of total Akt (B), P-Akt 473 (C) and P-Akt 308 (D) in muscles of WT and i6A− mice in the control (−SA) and both synergist ablation (+SA) groups. Values are means ± SEM, n = 7–8 and are the percentage of WT/−SA. *Different from WT/−SA (P < 0.05). **Different from WT/+SA (P < 0.05).
hindlimb muscle of i6A− mice in this study was 61% lower (P < 0.05) than that in muscle of WT mice [7.11 ± 0.25 nmol NADPH oxidized/(mg protein · min) vs. 18.26 ± 1.49 nmol NADPH oxidized/(mg protein · min)]. In contrast, TR1 activity was unaffected (data not shown). Western blot analysis confirmed that SelR and SelT levels were not different in skeletal muscle of WT and i6A− mice (data not shown). These results indicate that skeletal muscle differs from liver in terms of the relative effects of the mutant tRNA on selenoprotein translation, but consistent with the phenotypic studies, body weights, muscle size and fiber morphology were not different. Thus, skeletal muscles of i6A− mice exhibited no detectable phenotypic abnormalities with reductions in GPx-1 activity, but not SelR or SelT levels or TR1 activity.

In WT mice, 14 d after SA, plantaris mass in the +SA group was 1.9-fold greater than in the −SA group, similar to that reported previously (10,19). In contrast, the muscle mass...
from iA^- mice was increased 2.9-fold after SA (P < 0.01) (Fig. 1, for absolute mass data see Table 1). Histological examination indicated that the accelerated growth in the synergist-ablated muscles of iA^- mice was not associated with any inflammatory or pathological processes (Fig. 2). Protein concentrations of synergist ablated muscle homogenates did not differ between genotypes, indicating that the change in mass was a result of enhanced protein accumulation and was not associated with an edematous response (data not shown). Collectively these data indicate, by several independent criteria, that the enhanced growth in the +SA muscles of iA^- mice was the result of an acceleration of normal adaptation.

One means by which changes in selenoprotein levels could influence muscle adaptation is by affecting signal transduction pathways. Akt/PKB is a serine/threonine kinase that has recently been shown to be critical for muscle growth (9,10) and its activation can be inhibited by selenium-containing compounds (12,15). Activation of Akt can be detected by immunoblotting with phosphospecific antibodies that recognize phosphorylation at two amino acids, Thr^308 and Ser^473 (22). Muscles from nonsynergist ablated (-SA) iA^- mice had enhanced site-specific phosphorylation of Akt at Thr^308, but not at Ser^473, relative to control muscle from WT mice. The total amount of Akt was unaffected (Fig. 3). This was unexpected because numerous studies of insulin signaling in skeletal muscle have demonstrated that phosphorylation occurs at both Thr^308 and Ser^473 sites (22). Thus, increased phosphorylation at the Thr^308 site but not the Ser^473 site in the control plantaris muscles of iA^- transgenic mice is unique. In response to SA, both total Akt and Akt phosphorylation at Thr^308 and Ser^473 were significantly elevated in muscles of WT and iA^- mice, consistent with what was shown previously in mice after SA (10). These findings are noteworthy because they suggest that the normal signaling responses to SA were not changed in the muscles of the transgenic mice, but that the altered starting state of Akt in the muscles of iA^- mice may have contributed to the enhanced growth response.

Another candidate signaling molecule that has been associated with skeletal muscle growth (8,10) and its activation influenced by selenium is the ribosomal protein S6 kinase (p70S6k) (14). The regulation of p70S6k activation is complex, involving phosphorylation in the autoinhibitory domain at Thr/Ser^421/424 and the linker domain at Thr^389 (23,24). Like Akt, immunoblot analysis of p70S6k indicated enhanced site-specific phosphorylation at Thr/Ser^421/424 but not Thr^389 in -SA muscle from iA^- mice compared with that from WT mice (Fig. 4). Fourteen days after SA, muscles of WT and iA^- mice had significantly increased phosphorylation on both Thr/Ser^421/424 and Thr^389, but these changes did not differ between mice of the two genetic backgrounds (Fig. 4). Thus, as we concluded for Akt, iA^- expression does not alter the normal signaling for p70S6k after SA, suggesting that the increased site specific phosphorylation of both Akt and p70S6k in -SA muscle of iA^- mice underlies the enhanced adaptation to exercise. Preliminary data obtained in cultured cells in our laboratory indicated that GPx-1 levels influence the same
signaling pathways (Akt/p70S6k) that are altered in the muscles of i6A mice (unpublished observations). This provides some direction regarding potential selenoprotein targets and future studies will focus more specifically on the role GPx-1 in signaling and skeletal muscle adaptation.

Other signaling molecules have been implicated in the regulation of muscle adaptation after exercise, including p38 and ERK1/2 (7,25,26). The activities of p38 and ERK1/2 are enhanced by phosphorylation, and activation of these molecules can regulate a number of downstream effectors linked to transcription factors and protein synthesis (27,28). In contrast to Akt and p70S6k, both the phosphorylated and total amounts of these proteins did not differ between the −SA muscles of i6A and WT mice (Figs. 5, 6). Fourteen days after SA, there was a significant increase in the total amount of p38, but no increase in its phosphorylation. There was also a significant increase in the total amount of both ERK1 (44 kDa) and ERK2 (42 kDa). ERK1 showed enhanced phosphorylation (Fig. 6), but there were no differences between genotypes in −SA or +SA at 14 d.

Previous work by Bodine et al. (10) showed that the mTOR pathway is necessary for skeletal muscle growth after SA in mice. This kinase, mTOR, is downstream of Akt/PKB and upstream of p70S6k. Treatment of i6A mice with rapamycin completely blocked the growth associated with SA at 14 d (Fig. 7). These results are consistent with the findings of Bodine et al. (10) and indicate that the mTOR pathway is necessary for growth after SA. More importantly, these findings further confirm that the overexpression of tRNA_Ser(Sec) in the transgenic mice does not appear to alter the general mechanism of growth in response to SA but likely acts to enhance growth through subtle changes in the starting activation state at specific sites on Akt and p70S6k.

In summary, the findings from this study suggest that decreased levels of selective selenoproteins in skeletal muscle, achieved by expression of an altered tRNA_Ser(Sec), amplify the adaptive response to SA. This is the first indication that a single genetic manipulation can modify the magnitude of the adaptive response to exercise, with no additional side effects on adult muscle phenotype. Mechanistically, we suggest that decreased GPx-1 and/or other selenoprotein levels result in site-specific alterations of the intracellular signaling molecules, Akt and p70S6k, in the muscle of control sedentary transgenic mice. These subtle changes in the starting state of these growth-associated kinases do not alter control muscle phenotype, but are likely to enhance the signaling response to the exercise stimulus. Whether there are specific substrates for selenoproteins that are directly involved in these pathways, or whether altered selenoprotein levels alter the cellular environment (e.g., redox state), remains to be determined.

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LITERATURE CITED


