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学位論文の題目	Effects of long-term taurine supplementation on age-related changes in skeletal muscle function
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学位論文内容の要旨

Growing aging population becomes a major global concern, and this trend is expected to continue with a concurrent increase in age-related diseases. Aging, as an inevitable process, could lead to physiological functional decline in metabolic, respiratory, exercise capacity associated with loss of skeletal muscle mass and strength, obesity, and many metabolic diseases, such as type 2 diabetes. In skeletal muscle, aging process contributes to muscle weakness and increased fatigability, decline of skeletal muscle, mitochondrial function, and physical activity, leading to impaired mobility and loss of independence in individuals. Age-related declines in taurine content were observed in a number of tissues. So we speculate that taurine supplementation might have anti-aging effects on these age-related changes.

Taurine (2-aminoethanesulfonic acid) is a free amino acid found abundantly in mammalian tissues, particularly in excitable tissues such as the brain, cardiac, and skeletal muscles. From the perspective of food nutrition, taurine abounds in seafood and poultry. The most primary role of taurine is anti-fatigue. For this reason, taurine is added to various energy drinks, generally at a concentration of ~1000 mg per 250 mL serving. As an endogenous amino acid, taurine serves many physiological and pharmacological roles including: bile acids conjugation, plasma membrane stabilization, osmoregulation, neuromodulation, neurotransmission, and anti-oxidation. It was reported that taurine levels in muscle decreased after exercise, but taurine supplementation has effects on maintaining taurine level and improving exercise performance. Taurine is essential for skeletal muscle function. Taurine deficiency in taurine transporter knockout mice reduced the functioning of skeletal muscle. Additionally, taurine depletion accelerates skeletal muscle aging and leads to early death in mice. Chronic administration of taurine to aged rats improved the electrical and contractile properties of their skeletal muscle fibers. However, the anti-aging effects of taurine

and mechanism have not been clarified before. Given that taurine is associated with the function of skeletal muscle and exercise capacity as mentioned above, it is hypothesized that taurine might have anti-aging effects on age-related changes, especially skeletal muscle function.

In this study, the present author attempted to investigate age-related changes, including VO_2, spontaneous locomotor activity, and gene expression in skeletal muscle of aged rats from age of 32 to 92 weeks, which provide a potential perspective for taurine anti-aging research. With the aging of rats, VO_2 (mL/min/kg) in the resting, active, and whole day periods continuously decreased significantly from the age of 52 to 90 weeks relative to that at the age of 32 weeks, suggesting that the aging process might contribute to a decline in O_2 consumption, which is indicated as a typical age-related change. In the same way, with aging, the spontaneous locomotor activity in the resting, active, and whole day periods continuously decreased significantly from age 52 to 90 weeks relative to that at age 32 weeks. This indicates that the loss of spontaneous locomotor activity is an unavoidable phenomenon and a characteristic of the aging process. The mass of soleus muscle decreased significantly in rats aged 85 weeks (0.47 ± 0.04 g/kg) and 92 weeks (0.50 ± 0.06 g/kg) compared to that in rats aged 32 weeks (0.70 ± 0.03 g/kg). The mass of Gas muscle also decreased significantly in rats aged 85 weeks (5.00 ± 0.33 g/kg) and 92 weeks (5.62 ± 0.54 g/kg) compared to that in rats aged 32 weeks (8.89 ± 0.50 g/kg). The relative mitochondrial DNA level were decreased significantly in the soleus muscle of rats aged 85 and 92 weeks compared to that of rats aged 32 weeks. SDH staining of soleus muscle was performed to characterize mitochondrial enzyme function and myofiber oxidative capacity. From the results of SDH staining, the soleus muscle of rats aged 85 and 92 weeks showed a decrease in the positive staining level compared to that of rats aged 32 weeks. With aging, mRNA expression of myosin heavy chain 7 (MyHC-I) (*Myh7*), PGC-1α (*Ppargc1α*), cytochrome c (Cycs) (*Cycs*), and SDH (*sdha*) decreased significantly in the soleus muscle of rats aged 85 and 92 weeks compared to that of rats aged 32 weeks. Myosin heavy chain is an essential component of skeletal muscle and is associated with skeletal muscle contraction function. PGC-1α plays a major role in metabolic regulation, oxidative capacity, and regulation of respiration in skeletal muscle. Cycs is essential for energy production and mitochondrial respiration, as a component of the electron transport chain and as a mitochondrial marker protein. All these factors are skeletal muscle function related factor. The results of this study showed that the aging process might contribute to age-related decline of mRNA expression involved in mitochondrial oxidative function. Atrogin-1 and MuRF-1 have been identified as important enzymes in muscle atrophy, and regulation of their expression has the potential to prevent or reverse muscle atrophy in patients with sarcopenia. In this study, the relative expressions of MuRF1 (*Trim63*) and Atrogin-1 (*Fbxo32*) mRNA increased significantly in the soleus muscle of rats aged 85 and 92 weeks compared to that of rats aged 32 weeks. Aging is also associated with the dysregulation of lipid metabolism. In this study, the weight of WAT around the kidney was increased significantly in rats aged 85 and 92

weeks compared to that in rats aged 32 weeks. Oil-Red O staining results showed that lipid accumulation was also increased in the soleus muscle of SD rats aged 92 weeks compared to that of rats aged 32 weeks. Plasma FFA and cholesterol increased as the rats aged. It is suggested that the aging process might contribute to the decline of muscle mass, and to the increase in lipid accumulation in the body.

Although there are numerous researches of taurine effects, most of them focused on supplementation of relatively high concentrations of taurine, or short-term taurine supplementation models. The research of taurine effects of a relatively low dose, long-term administration is limited. To know the effects of low dose of taurine supplementation, just like daily food intake, the present author attempted to examine the anti-aging effects of long-term administration of 0.5%, or 1% taurine from age of 32 to 56 weeks on age-related changes in VO_2 consumption, spontaneous locomotor activity, and skeletal muscle function. VO_2 in the water group during the active period and the whole day period continuously decreased significantly every month as the rats aging, however, VO_2 in the 0.5% and 1% taurine groups did not decrease significantly until 49 or 53 weeks. This indicates that long-term and relatively low dose of taurine could attenuate the age-related changes in O_2 consumption. In the same way, in the active and whole day periods, spontaneous locomotor activity of the water group decreased significantly every month as rats aged, whereas in the 0.5% taurine group, it started to decrease significantly from age 41 weeks (active period) and 45 weeks (the whole day). Additionally, in the 1% taurine group, the spontaneous locomotor activity did not decrease significantly until 49 weeks (both in the active and the whole day periods) relative to that at the age of 33 weeks. This suggests that long-term administration of taurine at relatively low dose could attenuate the age-related change in the spontaneous locomotor activity. Plasma taurine levels were significantly higher in the 0.5% and 1% taurine groups than in the water group at 37, 41, and 53 weeks of age. The level of taurine was measured in the Gas muscle, extensor digitorum longus (EDL) muscle, soleus muscle, intestine, liver, and kidney in 24 h after administration of taurine. Twenty-one week after administration, at the age of 56 weeks, the level of taurine (μmol/g) in the 1% taurine group was the highest in the kidney and intestine among the three groups. There was no significant change in taurine level among groups in each skeletal muscle after the long-term administration of taurine. To determine the taurine uptake by the skeletal muscle tissue, taurine incorporation into L6 myotube cells was measured. Treatment with taurine (300 μM) for 120, 180, and 240 min increased the incorporation of taurine into L6 cells in a time-dependent manner. From the results of taurine incorporation into L6 cells in this study, taurine supplementation might promote the incorporation of taurine into skeletal muscles, which might have an effect on the physiological functions.

To examine the mechanism of long-term administration of taurine on modulation of molecules related to energy metabolism in skeletal muscle, the phosphorylation of AMPK in soleus and Gas

muscles was determined. Phosphorylated AMPK in soleus muscle tended to increase in both taurine groups. In Gas muscle, the level of phosphorylated AMPK was significantly increased in the 1% taurine group. AMPK plays a key role in the regulation of energy metabolism, oxidative capacity, exercise capacity, and aging processes. AMPK also has a role in stimulation of glucose uptake in skeletal muscle by induction of primary glucose transporter, GLUT4 gene via HDAC4/5-MEF2 axis, in which the transcription of GLUT4 gene is modulated by MEF2A, a member of the MEF2 family of transcription factors, and the transcriptional activity of MEF2A is enhanced by AMPK. In the present study, plasma glucose levels in the 0.5% and 1 % taurine groups were significantly lower at 56 weeks than in the water group. And the expressions of *GLUT4* gene and protein were significantly induced in the Gas muscle of rats in the 1% taurine group. In 0.5% taurine group, it shows a significant increase of *GLUT4* gene and an increase tendency of GLUT4 protein in Gas muscle. Furthermore, the expression of MFE2A protein was significantly increased in 1% taurine group in Gas muscle. Taurine group of 0.5% shows significant increases in *MEF2A* gene and protein in Gas muscle. These results indicate that taurine supplementation might contribute to increase glucose uptake in skeletal muscle cells and lower plasma glucose levels through the induction of MEF2A and GLUT4 via the function of AMPK. Aging is associated with dysregulation of energy metabolism and mitochondrial dysfunction. *SDH* gene expression in the soleus and Gas muscles of taurine groups tended to be higher than that in the water group. SDH activity in Gas muscle also result significantly higher in both taurine groups. Taurine supplementation increased *Cycs* mRNA expression levels in the soleus muscle of the 0.5% taurine group and in the Gas muscle of 1% taurine group. These indicate that supplementation of taurine might improve the age-related decline in mitochondrial oxidative capacity. PGC-1α plays a key role in the regulation of mitochondrial biogenesis and oxidative metabolism, its activity is regulated by AMPK. In this study, the expression of PGC-1α protein tended to be increased in Gas muscle of 1% taurine group. *Cycs* and *GLUT4* genes were increased significantly in 1% taurine group. All these factors are associated with the function of mitochondria and skeletal muscle, and it is indicated that the function of skeletal muscle would be improved by long-term taurine supplementation at relatively low dose during aging process.

This study contributes to the further understanding of age-related changes and examines of the mechanism underlying the effects of taurine on age-related changes, which may have potential clinical implications. As an endogenous amino acid, taurine may be an ideal treatment for daily intake or for further testing in clinical trials. A more extensive investigation on skeletal muscle cells, animal models, or human models is needed to further explore its potential application in clinical trials for coping with the concern of a rapidly growing aging population.

主業績

No.1	
論文題目	Effects of long-term taurine supplementation on age-related changes in skeletal muscle function of Sprague-Dawley rats
著者名	Yun Ma, Hitomi Maruta, Baojun Sun, Chengduo Wang, Chiaki Isono, Hiromi Yamashita
発表誌名	Amino Acids, 53(2) 159-170 (2021)

副業績

No.1	
論文題目	Age-related changes in energy metabolism and skeletal muscle function of Sprague-Dawley rats
著者名	Yun Ma, Hitomi Maruta, Baojun Sun, Hiromi Yamashita
発表誌名	岡山県立大学保健福祉学紀要、第 27 巻 1 号, p.83-92, 2020 年
No.2	
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著者名	
発表誌名	

関連業績

No.1	
論文題目	Seasonal variations in major components of Crassostrea gigas from Seto Inland Sea
著者名	Chiaki Isono, Hitomi Maruta, Yun Ma, Natsuki Ganeko, Tsuyoshi Miyake, Hiromi Yamashita
発表誌名	Fisheries Science, 86(6) 1087-1099 (2020)
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論文題目	
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論文審査結果の要旨

タウリンは、骨格筋機能の維持および運動能力の亢進に関連した因子であることが示されてきているが、これまでの研究は高用量のタウリン補給の影響について調べた研究が多かった。通常食品から摂取するタウリンの量は低用量である。申請者はタウリンが AMP 活性化プロテインキナーゼ（AMPK）を活性化するとの報告に着目し、低用量のタウリンであっても長期間摂取することにより AMPK の活性化を介して、加齢によるエネルギー代謝低下および骨格筋機能低下を改善できるのではないかと推測した。AMPK は脂質代謝やグルコースの取り込みの調節、持久運動能力、抗老化などに関連する因子である。本論文は、長期間低用量のタウリン摂取が加齢によるエネルギー代謝の低下および骨格筋機能の低下に対して改善効果を示すか検討したものである。先ず加齢によるエネルギー代謝ならびに骨格筋機能の変化について把握するために、32 週齢から 92 週齢に至る過程での SD ラットの酸素消費量、自発運動量、筋組織重量、および血液生化学検査値、および骨格筋の機能に関連した遺伝子発現の変化を解析した。その結果、加齢により酸素消費量および自発運動量が低下した。また骨格筋重量の低下、ならびに骨格筋の機能および酸化能力に関連する数種の因子、およびミトコンドリ DNA がヒラメ筋で有意に低下した。これより、加齢によりエネルギー代謝能力および骨格筋機能が低下すると示唆された。次に SD ラットを水群（対照群）、0.5%タウリン補給群、および 1%タウリン補給群の 3 群に分けて、それぞれ水、0.5%（25mg/kg 体重/日）および 1%（50mg/kg 体重/日）のタウリンを 34～56 週齢の間投与した。その結果、比較的低用量のタウリンを長期間補給することにより、加齢による酸素消費量および自発運動量の低下が抑制される傾向が見られた。またタウリン補給により AMPK の活性化を介して、骨格筋機能に関連した因子の発現が増加し、エネルギー代謝能力や骨格筋機能の改善につながると示唆された。

以上の結果より、学術上、実際上寄与するところが少なくない。よって、本論文は博士（栄養学）の学位論文として価値あるものと認める。