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| 授与した学位 | 博　士 |
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| 学位論文の題目 | Study on the Physiological Function of Taurine in Skeletal Muscles |
| 学位審査委員会 | 主査　山下　広美　副査　伊東　秀之　副査　川上　貴代<br>副査　住吉　和子　副査　岩満　賢次 |

## 学位論文内容の要旨

Taurine (2-aminoethanesulfonic acid) is a free amino acid abundantly found in mammalian tissues, particularly in excitable tissues, such as the brain, retina, heart, and skeletal muscles. Taurine is either obtained through diet, such as seaweed, seafood and meat, or synthesized from cysteine in the body. Taurine serves a physiological and pharmacological role including: osmoregulation, cell membrane stabilization, anti-inflammatory effects, mitochondrial tRNA activities, and calcium homeostasis. Taurine is essential for skeletal muscle function. Taurine deficiency in taurine transporter knockout mice reduced the skeletal muscle functions. Taurine affects the skeletal muscle contraction and enhances exercise performance by inhibiting oxidative stress in rats. It was reported that taurine supplementation has effects on maintaining taurine level and improving exercise performance. However, only a few studies have described how taurine levels change in skeletal muscles after oral administration and its specific roles in skeletal muscle function. Taurine may have an important role in muscle function and may control muscle metabolism and gene expression; however, the specific action mechanisms remain unclear.

In our previous study, we investigated the effect of long-term taurine supplementation on age-related changes in skeletal muscle function and found that long-term taurine supplementation at a relatively low dose (0.5% and 1% taurine) modulates age-related changes in respiration, metabolism, and skeletal muscle function. In this study, to investigate the effects of taurine on skeletal muscle in more detail, we designed the animal experiments with a short-term taurine supplementation based on the previous long-term taurine supplementation at a relatively low dose experimental in SD rats, and the cell experiments with skeletal muscle cells. The present author attempted to investigate the changes in taurine levels in blood and skeletal muscles after oral administration, the beneficial roles of taurine, and its mechanism of action on skeletal muscle functions in SD rats and L6 myotubes.

The changes of taurine concentration in plasma and skeletal muscles, were measured from 1 to 4 h after the oral administration of taurine to rats at 14-weeks-of-age. Plasma taurine concentration was significantly increased at 1 h after the administration of taurine in a dose-dependent manner compared to that in the water group. Taurine concentrations in the soleus muscle of both taurine groups (0.5% and 1% taurine) were significantly increased at 2 h and those in the 0.5% taurine group at 3 h and in the 1% taurine group at 4 h were significantly increased compared to those in the water group. Taurine concentrations in the plantaris muscle of both taurine groups were significantly increased at 2 and 3 h compared to those in the water group. It clearly suggested that Taurine may be absorbed into the blood after administration and then transported to the skeletal muscles within 2 h. The taurine content in skeletal muscles may return to basal levels 4 h after administration. Taurine concentrations in the GAS and TA muscles of both taurine groups showed a tendency to increase but not statistically significant. Sved et al. reported the concentration of taurine in plasma and tissues after dosing $^{14}$C-taurine in rats. They describe that the rate of elimination of intracellular taurine will depend on the rate of turnover of the intracellular pool for that particular tissue. Taurine level in the muscles may depend on taurine absorption and processing capacity.

The effects of relatively low-dose and short-term taurine (10 days) supplementation on the expression of genes associated with skeletal muscle function such as respiratory metabolism and mitochondrial function were analyzed after taurine administration. Expression of the MEF2A and Cycs genes were significantly increased in the GAS muscle of the 1% taurine group, and the SDH gene was increased in the GAS muscle of both taurine groups compared to that in the water group. In the soleus muscle, MEF2A, PGC-1α, SDH, Cycs, myoglobin, and TauT genes were increased in both taurine groups, and the GLUT4 gene expression was increased in the 1% taurine group compared to that in the water group. In the plantaris, MEF2A and SDH genes were increased in both taurine groups, and the myoglobin gene was increased in the 1% taurine group compared to that in the water group. In TA muscle, SDH, Cycs, and TauT genes were increased in both taurine groups, and MEF2A, PGC-1α, and myoglobin genes were higher in the 1% taurine group than in the water group. This suggested that taurine supplementation was significantly increased the expression of genes associated with respiratory metabolism and mitochondrial function.

Previously, long-term administration of taurine at a relatively low dose attenuates the age-related decline in $O_2$ consumption and spontaneous locomotor activity with the activation of AMP-activated protein kinase (AMPK). AMPK is a sensor of cellular energy status and plays a key role in the regulation of energy metabolism, oxidative capacity, and exercise capacity. To determine whether the function of taurine is associated with AMPK phosphorylation in the skeletal muscle of SD rats at 14-weeks-of-age, phosphorylated AMPK was analyzed in skeletal muscles after taurine administration. The phosphorylation of AMPK in the GAS, soleus, and TA

muscles of rats administered taurine was significantly increased compared to that in rats in the control group. This suggests that the effect of taurine on the muscles is independent of the level of taurine in the muscles. AMPK is a key mediator of cell signaling pathways intrinsically linked to muscle function and metabolism. Activation of AMPK can increase mitochondrial enzymes in skeletal muscles. MEF2A is a member of the MEF2 family of transcription factors involved in skeletal muscle differentiation and is regulated by AMPK. The expression levels of MEF2A gene were significantly increased in the four skeletal muscles of the 0.5% and 1% taurine groups depending on the muscles. The expression levels of MEF2A protein were also increased in the GAS, soleus, and TA muscles of the 0.5% and 1% taurine groups. Both MEF2 and AMPK are involved in the regulation of GLUT4 gene transcription. GLUT4 is a glucose transporter protein. The expression of GLUT4 gene was significantly increased in the soleus muscle of the 1% taurine group. PGC-1α gene and protein expressions in the soleus muscle were significantly increased in the 1% taurine group. PGC-1α is a transcriptional coactivator that plays a key role in the regulation of mitochondrial biogenesis and oxidative metabolism and its activity is regulated by AMPK. The mRNA expression levels of SDH, which is a marker enzyme of mitochondria, were significantly increased in the four kinds of skeletal muscles of both taurine groups 4 h after the administration of taurine compared to those in the control group. SDH staining levels of the GAS and TA muscles in both taurine groups were significantly higher than those in the control group. The expression levels of cytochrome c, which is a component of the electron transport chain of mitochondria, a marker of mitochondrial biogenesis, were also higher in the GAS, soleus, and TA muscles of the 0.5% and 1% taurine groups than in the control group. In addition, MEF2A and PGC-1α are also involved in the expression of myoglobin, which is an essential oxygen-storage hemoprotein that facilitates oxygen transport and is required for lipid and glucose oxidation within skeletal muscles. The expression levels of myoglobin gene were significantly increased in the soleus, plantaris, and TA muscles of the 0.5% and 1% taurine groups compared to those in the control group. The expression levels of myoglobin protein were significantly increased in the GAS, soleus, and TA muscles of the 0.5% and 1% taurine groups compared to those in the control group. These results suggest that taurine supplementation increases mitochondrial biogenesis and improves oxidation capacity associated with skeletal muscle function through these molecules regulated by the activation of AMPK.

However, how taurine activates AMPK remain unclear, the mechanism underlying taurine function in skeletal muscles has still not clear. Numerous studies have showed that $Ca^{2+}$ activates AMPK by activating CaMKK. It was reported that the presence of taurine can increase the release of $Ca^{2+}$ from the sarcoplasmic reticulum. Taurine could regulate intracellular $Ca^{2+}$ levels, but the mechanisms remain unclear. Phospholipase C (PLC) is a class of membrane-associated enzymes that is involved in the regulation of $Ca^{2+}$. Activated PLC increases the inositol-1,4,5-triphosphate

($IP_3$) levels and stimulates $IP_3$ receptor on the endoplasmic reticulum membrane, and then releases calcium influx into cells. Taurine supplementation increased the $Ca^{2+}$ handling, which possible involvement of PLC. So we speculate that taurine supplementation might activate PLC to release $IP_3$, which induces calcium influx into cells, and then activates AMPK.

To further investigate the mechanism of taurine activation of AMPK in the skeletal muscle, the study was conducted by L6 cell model. Treatment with taurine (0.3 mM), which is near the plasma taurine level, increased the expression levels of myogenic genes associated with mitochondrial function and respiratory metabolism, as shown in the skeletal muscles of SD rats. Taurine treatment stimulated the phosphorylation of AMPK and increased the expression levels of MEF2A, PGC-1α, myoglobin, and GLUT4 proteins. To elucidate the signaling pathway of taurine, we used antagonist of taurine transporter, GES, AMPK inhibitor, araA, and PLC inhibitor, YM-254890 (YM). The enhancing effects of taurine on the phosphorylation of AMPK and the expression of myogenic genes and proteins were completely suppressed by GES treatment. This suggests that taurine performs its physiological function when it enters the cells through the taurine transporter. As for the treatment with the AMPK inhibitor araA, the effects of taurine on the phosphorylation of AMPK and on the expression of myogenic genes and proteins were also suppressed. This indicates that taurine performs its physiological function by activating AMPK and stimulating its downstream factors, MEF2A, PGC-1α, myoglobin, and GLUT4. Taurine treatment stimulated calcium influx, but the PLC inhibitor, YM, inhibited this stimulation. In addition, after treatment with YM, the effects of taurine on the phosphorylation of AMPK and the expression of myogenic genes and proteins were completely suppressed. Activated PLC increases the $IP_3$ levels and induces calcium influx in cells. This indicates that taurine can stimulate calcium influx via PLC, and $Ca^{2+}$ can activate AMPK by activating CaMKK. However, further studies are required to determine whether TauT is coupled with G-protein and the mechanism by which taurine associates with PLC. The data obtained here prompt the suggestion that taurine entered cells through the taurine transporter and activated PLC, released calcium influx into cells, and then performed its physiological function by activating AMPK and stimulating its downstream factors, MEF2A, PGC-1α, myoglobin, and GLUT4.

Collectively, this study demonstrates that taurine can stimulate PLC to increase the calcium influx in the cells via the interaction with the taurine transporter, thereby activating AMPK. Through the PLC–$Ca^{2+}$–AMPK signaling pathway, the expression levels of genes and proteins associated with the key factors, MEF2A and PGC-1α, are increased, along with the expression levels of GLUT4, myoglobin, and mitochondrial proteins, SDH and Cycs. Our findings provide insights into the role of taurine in improving the skeletal muscle function.

主業績

| No.1 | |
|---|---|
| 論文題目 | Taurine Stimulates AMP-Activated Protein Kinase and Modulates the Skeletal Muscle Functions in Rats via the Induction of Intracellular Calcium Influx |
| 著者名 | Baojun Sun，Hitomi Maruta，Yun Ma，Hiromi Yamashita |
| 発表誌名 | International Journal of Molecular Sciences (2023，24.4: 4125.)<br>DOI: 10.3390/ijms24044125 |

副業績

| 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, (2021，53: 159-170.).<br>DOI: 10.1007/s00726-020-02934-0. |
| No.2 | |
| 論文題目 | |
| 著者名 | |
| 発表誌名 | |

関連業績

| 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 2020 年 |
| No.2 | |
| 論文題目 | |
| 著者名 | |
| 発表誌名 | |

# 論文審査結果の要旨

本論文は、タウリン投与後動物骨格筋中のタウリン含有量の変化及び骨格筋への影響を検討すると共に、タウリン処理による骨格筋細胞への影響について検討し、タウリンの骨格筋における作用について明らかにすることを目的として研究した結果をまとめたものであり、得られた成果は次のとおりである。

1. 11 週齢 SD ラットを、水群、0.5%タウリン群、および 1%タウリン群に分け、2 週間の予備飼育後に継続的に 10 日間タウリンをゾンデにより投与し、10 日目の投与後に解剖を行った。タウリンを投与したラットの血漿タウリン濃度は投与後 1 時間後に有意な増加がみられた。骨格筋のタウリン含有量は、投与 2 時間および 3 時間後に増加の傾向が見られたが、4 時間後にはほぼ水群のレベルに戻った。骨格筋におけるタウリン増加のレベルは骨格筋の種類により異なっていた。

2. 骨格筋における呼吸代謝関連遺伝子の発現を解析した結果、MEF2A、SDH 遺伝子を中心に有意な増加が見られた。またヒラメ筋、腓腹筋、前脛骨筋では、呼吸代謝関連タンパク質の発現、およびその発現に関わるリン酸化 AMPK が増加していた。SDH 染色、ミトコンドリア DNA 量の解析より、腓腹筋、前脛骨筋ではミトコンドリア増幅が示唆された。

3. 5%$CO_2$、37°C の条件下で L6 筋芽細胞を培養し、筋管細胞へ分化誘導を行った。分化開始後 11 日目に実験を行った。L6 筋管細胞においてもタウリン処理後に呼吸代謝関連遺伝子、リン酸化 AMPK ならびに関連タンパク質の発現が有意に増加した。各種阻害剤を用いて、タウリンの骨格筋における作用のメカニズムを検討した結果、タウリンはタウリントランスポーター介して細胞内に取り込まれる際に細胞内のカルシウム流入を刺激し、それによる AMPK の活性化、さらに AMPK の活性化を介して呼吸代謝関連の遺伝子発現を増加させると示唆された。

以上より、一定量のタウリンの継続的な摂取は骨格筋の酸化能力を増加させ、骨格筋機能を改善すると示唆された。タウリンを含む食品の摂取による同様な機能性も期待できる。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。