Doctoral Dissertation

Effects of long-term taurine supplementation on age-related changes in skeletal muscle function

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YUN MA

The Graduate School of Health and Welfare Science

Okayama Prefectural University

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Abbreviations

SD	Sprague-Dawley
AMPK	AMP-activated protein kinase
SDH	succinate dehydrogenase
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Cycs	cytochrome *c*
MEF2A	myocyte enhancer factor 2A
GLUT4	glucose transporter 4
MyHC-I	myosin heavy chain 7
MuRF1	Muscle RING-finger 1
Gas	gastrocnemius
EDL	extensor digitorum longus
WAT	white adipose tissue
FFA	free fatty acids
H&E	hematoxylin and eosin

Chapter I Introduction

There is no doubt that the rapid growth in aging population becomes a major global concern, and this trend is expected to continue with a concurrent increase in age-related diseases [1]. 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 [2-4]. Maximal oxygen uptake (VO_2max) declines with age, possibly due to a diminished capacity to deliver and extract O_2 during exercise, in humans [5, 6]. In skeletal muscle, aging process contributes to muscle weakness and increased fatigability [7], decline of skeletal muscle, mitochondrial function, and physical activity [8], leading to impaired mobility and loss of independence in individuals [9]. Dawson reported that significant age-related declines in taurine content were observed in a number of tissues with advancing age [10]. 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 [11], particularly in excitable tissues such as the brain, cardiac, and skeletal muscles [12,13]. From the perspective of food nutrition, taurine abounds in seafood and poultry [14]. 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 [15,16]. As an endogenous amino acid, taurine serves many physiological and pharmacological roles including: bile acids conjugation [17], plasma membrane stabilization [18], osmoregulation [19], neuromodulation [20], neurotransmission [21], and anti-oxidation [22]. It was reported that taurine supplementation could improve exercise performance and duration, up-regulated physical endurance, and maintained the concentration of taurine in the muscle [23,24]. Taurine levels in muscle decreased after

exercise, but taurine supplementation has effects on maintaining taurine level and improving exercise performance [25,26]. Taurine is essential for skeletal muscle function. Warskulat et al. (2004) found that taurine deficiency in taurine transporter knockout (taut−/−) mice reduced the functioning of skeletal muscle [27]. Additionally, taurine depletion accelerates skeletal muscle aging and leads to early death in mice [28]. Chronic administration of taurine to aged male Wistar rats improved the electrical and contractile properties of their skeletal muscle fibers [29]. 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 (the detail shown in Chapter II), which provide a potential perspective for taurine anti-aging research. In addition, 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. In order 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 (5 months) administration of 0.5% or 1% taurine on age-related changes (the detail shown in Chapter III) in VO_2 consumption, spontaneous locomotor activity, skeletal muscle function. The evidence of this study could be of potential clinical value for aging individuals.

Chapter II

Age-related changes in energy metabolism and skeletal muscle function of Sprague-Dawley rats

Aging is an unavoidable process that causes a decline in metabolic, respiratory, and exercise functions, which are associated with loss of skeletal muscle mass and strength, mitochondrial dysfunction, and metabolic diseases [2-4, 30]. Aging decreases skeletal muscle and mitochondrial function and leads to a reduction in their functional capacity, which results in a decline in physical activity [7, 8]. Evidence shows that maximal oxygen uptake (VO_2max) declines with age [5, 31-32]. In one study, it was found that VO_2max was 36% lower in master runners than in young runners, possibly as a consequence of diminished capacity to deliver and extract O_2 during exercise [6]. Conley et al. [33] reported a 50% reduction in oxidative capacity in older human subjects compared to young subjects, due to reduced mitochondrial volume and reduced mitochondrial function. Previous studies reported that the level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which is a transcriptional coactivator that regulates the genes involved in energy metabolism and is the primary regulator of mitochondrial functions [34-36], in skeletal muscle decreased with age [37]. Aging process is also associated with dysregulation of lipid metabolism. Honma showed that the weight of white adipose tissue (WAT) and levels of liver triacylglycerol, plasma free fatty acids (FFA), and plasma insulin showed an increase with aging in SAMP10 mice [38]. Muscle RING-finger 1 (MuRF1), which is highly induced in atrophying muscle, may play an important role in the breakdown of myofibrillar proteins [39, 40]. Atrogin-1 mRNA increases 8 to 40-fold in atrophy study, and this increase precedes the onset of muscle weight loss [39, 41]. In Chapter II, age-related changes was investigated, including VO_2, spontaneous locomotor activity, tissue weight, blood biochemical index, and gene expression in skeletal muscle of Sprague-Dawley (SD) rats aged 32 to 92 weeks.

MATERIALS AND METHODS

Materials

Formalin, 2-mercaptoethanol, 1% eosin Y solution, and commercial assay kit for free fatty acid (FFA) (NEFA C-Test Wako), cholesterol (Cholesterol E-Test Wako) and Oil-Red O were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Ethanol and nitro blue tetrazolium were purchased from Sigma-Aldrich (St. Louis, MO, USA), and hematoxylin from Merck KGaA (Darmstadt, Germany). Mount-Quick and Mount-Quick aqueous mounting media were purchased from Daido Sangyo Co., Ltd (Tokyo, Japan). Sepasol-RNA I Super G and sodium succinate were purchased from Nacalai Tesque (Kyoto, Japan). RNase inhibitor, ReverTra Ace® qPCR (quantitative polymerase chain reaction) Master Mix, and gDNA (genomic DNA) remover kit were purchased from TOYOBO Co., Ltd. (Osaka, Japan). KAPA SYBR® FAST qPCR kit was purchased from Kapa Biosystems (Wilmington, MA, USA).

Animal experiments

The care and use of the animals in this study followed the guidelines of Okayama Prefectural University and the laws and notifications of the Japanese government. All animal experiments were approved by the Animal Care and Use Committee of Okayama Prefectural University (protocol number 27-3). Male SD rats aged 32 weeks (n=4-15) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were housed individually in an air-conditioned room at 25 °C with an alternating 12 h light/dark cycle (light, 8:00-20:00). All rats were allowed free access to commercial food (CE-2, CLEA Japan, Inc., Tokyo, Japan) and water. Food intake and body weight were measured daily. Respiratory metabolism was measured with VO_2 and CO_2 generation

volumes, using a metabolism measuring system (MK-5000, Muromachi Kikai, Tokyo, Japan), and spontaneous locomotor activity was measured using an infrared sensor (SUPERMEX SENSOR PYS-001, Muromachi Kikai, Tokyo, Japan). Each rat was kept in a sealed chamber for 24 h at 25 °C. The airflow was 3.0 L/min. The animals had free access to water and food. At the age of 32, 85, and 92 weeks, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (100 µL/100 g of body weight). Tissue were collected and stored at -80 °C until subsequent measurements. Blood samples were collected in heparinized tubes and centrifuged at 3,000 × g and 4 °C for 10 min and plasma was obtained. FFA and cholesterol in plasma were determined by commercial kit.

Histological analysis

The soleus muscle was cut into 10 µm sections using a Leica CM3050 S cryostat (Leica Microsystems, Wetzlar, Germany) at -20 °C and fixed in 10% (v/v) formalin. The sections were stained with hematoxylin and eosin (H&E) and mounted in Mount-Quick medium. The sections were stained with Oil-Red O and mounted in Mount-Quick Aqueous medium. For succinate dehydrogenase (SDH) staining, the soleus sections were air-dried at room temperature and incubated in a solution consisting of 50 mM phosphate buffer (6 mM potassium dihydrogenphosphate and 44 mM disodium hydrogenphosphate), 50 mM sodium succinate, and 0.5 mg/mL nitro blue tetrazolium at 37 °C for 40 min. The sections were washed in double distilled H_2O for 3 min and mounted in Mount-Quick aqueous mounting medium. Images were captured with a CCD camera (Olympus Optical, Tokyo, Japan) at ×100 magnification.

qRT-PCR analysis

Total RNA was isolated from frozen tissue with Sepasol-RNA I Super G. RNase inhibitor was added according to the manufacturer's instructions. Total RNA was measured and cDNA was prepared using the ReverTra Ace® qPCR Master Mix and gDNA remover kit. After RNA extraction with Sepasol RNA I Super G, DNA was obtained by 250 µl back extraction buffer (4 M guanidine thiocyanate; 50 mM sodium citrate; 1 M Tris, pH 8.0) into the phenol phase and interphase. qRT-PCR was performed in a StepOnePlus real-time PCR system (Applied Biosystems, CA, USA) using the KAPA SYBR® FAST qPCR kit, to evaluate specific mRNA content. Data were normalized to β-actin mRNA, and the expression levels were compared to the mRNA expression values in rats aged 32 weeks. The qRT-PCR primers are listed in Table 1.

Statistical analysis

Data are presented as mean ± standard error (SE). Results were analyzed using Student's T-test or subjected to one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test (Statcel4, OMS Inc., Tokyo, Japan).

Table 1. Primers used for PCR.

Gene	Direction	Primer sequence
β-actin (*Actb*)	forward	5′ -GGAGATTACTGCCCTGGCTCCTA-3′
	reverse	5′ -GACTCATGTACTCCTGCTTGCTG-3′
MyHC-I (*Myh7*)	forward	5′ -AGAGGAAGACAGGAAGAACCTAC-3′
	reverse	5′ -GGCTTCACAGGCATCCTTAG-3′
PGC-1α (*Ppargc1α*)	forward	5′ -GACCCCAGAGTCACCAAATGA-3′
	reverse	5′ -GGCCTGCAGTTCCAGAGAGT-3′
Cycs (*Cycs*)	forward	5′ -AGCGGGACGTCTCCCTAAGA-3′
	reverse	5′ -CTTCCGCCCAAACAGACCA-3′
SDH (*sdha*)	forward	5′ -TGGGGCGACTCGTGGCTTTC-3′
	reverse	5′ -ATCTCCAGTTGTCCTCTTCCA-3′
MuRF1 (*Trim63*)	forward	5′ -CGACTCCTGCCGAGTGACC-3′
	reverse	5′ -GCGTCAAACTTGTGGCTCAG-3′
Atrogin-1 (*Fbxo32*)	forward	5′ -GAACAGCAAAACCAAAACTCAGTA′
	reverse	5′ -CTCCTTAGTAGTACTCCCTTTGTGAA-3′
MtND1 (*mt-Nd1*)	forward	5′ -CTCCCTATTCGGAGCCCTAC-3′
	reverse	5′ -ATTTGTTTCTGCTAGGGTTG-3′

RESULTS

Age-related changes of body weight gain, respiratory metabolism, and spontaneous locomotor activity

To see the effect of aging process on the respiratory metabolism and spontaneous activity, VO2 and spontaneous locomotor activity were measured for SD rats from 32 weeks to 90 weeks of age. As rats aging, VO_2 (mL/min/kg) and spontaneous locomotor activity (count/min) during the resting period, active period, and the whole day period significantly decreased continuously from age 52 weeks (52 w) to 90 weeks (90 w) compared with that at 32 weeks (32 w) (Fig 1A, B). The body weight gain (g) increased gradually as rats aging, from age 34 to 91 weeks (Fig 1C).

Age-related changes of lipid accumulation

As shown in Table 2, weight of WAT around the kidney increased significantly in the rats aged 85 weeks (38.8 ± 3.35 g/kg) and 92 weeks (38.3 ± 2.19 g/kg) compared to that in rats aged 32 weeks (19.4 ± 1.86 g/kg). The weight of WAT around the testicle increased, but not statistically significant, in rats aged 85 and 92 weeks, compared to that in rats aged 32 weeks. 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). As rats aged, the level of plasma FFA (mEq/L) increased significantly in rats aged 50 to 92 weeks compared to that in rats aged 32 weeks (Fig 2A). The level of plasma cholesterol (mg/dL) tended to increase in rats during the

aging process (Fig 2B). The results of H&E staining and Oil-Red O staining, age-related changes in the soleus muscle in SD rats, are shown in Fig 3. The tissue sections with Oil-Red O staining in rats aged 85 weeks showed more lipid accumulation and it of 92 weeks showed a marked increase in lipid accumulation compared to that in rats aged 32 weeks.

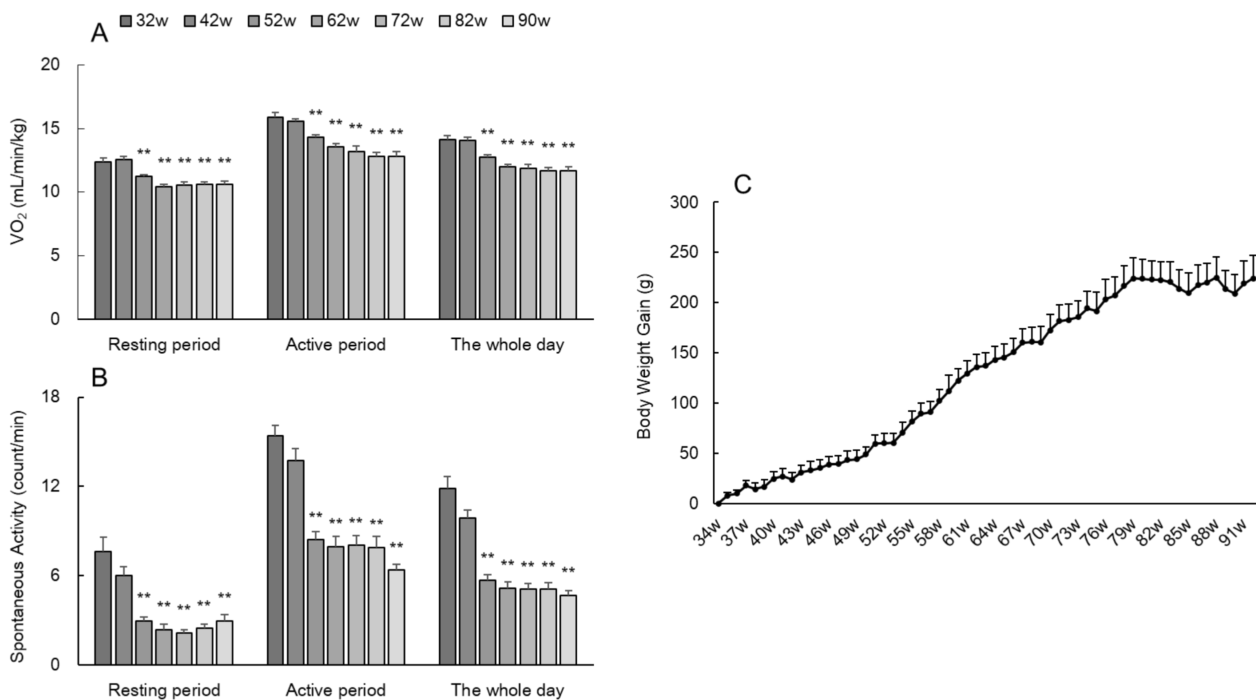

Fig 1. VO_2 in SD rats from 32 to 90 weeks of age in resting, active, and whole day periods (a). Spontaneous locomotor activity of SD rats from 32 to 90 weeks of age in the resting, active, and whole day periods (b). The resting period was 10:00 am–8:00 pm, active period was 8:00 pm–8:00 am, and the whole day period was from 10:00 am–8:00 am. Average body weight gain in SD rats from 34 to 91 weeks of age (c). Values shown represent means ± SE for 4–15 rats. *$p < 0.05$, **$p < 0.01$ statistically significant versus the value obtained at 32 weeks of age. Results were analyzed with one-way ANOVA followed by the Tukey–Kramer post hoc test for multiple comparisons

Table 2. Aging-related changes on tissue weight of WAT (kidney), WAT (testicle), soleus muscle and Gas muscle in SD rats at age 32, 85 and 92 weeks.

g/kg-BW	32w	85w	92w
WAT (kidney)	19.4 ± 1.86 [a]	38.8 ± 3.35 [b]	38.3 ± 2.19 [b]
WAT (testicle)	20.9 ± 1.19 [a]	22.5 ± 1.48 [a]	25.1 ± 1.04 [a]
Soleus	0.70 ± 0.03 [a]	0.47 ± 0.04 [b]	0.50 ± 0.06 [b]
Gas	8.89 ± 0.50 [a]	5.00 ± 0.33 [b]	5.62 ± 0.54 [b]

Values shown represent means ± SE of 3-7. Results were analyzed with one-way ANOVA followed by the Tukey-Kramer post hoc test for multiple comparisons. Groups without the same letter are significantly different (p<0.05).

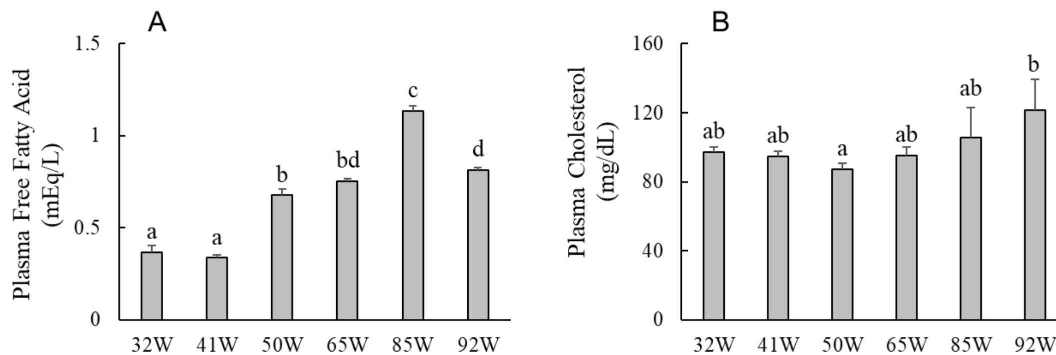

Fig 2. Age-related changes in plasma FFA and cholesterol in SD rats aged 32 to 92 weeks. (A) Plasma FFA level (mEq/L), (B) Plasma cholesterol level (mg/dL). The values shown represent mean ± SE of 3-12 rats. The results were analyzed with one-way ANOVA followed by the Tukey-Kramer post-hoc test for multiple comparisons. The groups without the same letter represent a statistically significant difference (p<0.05).

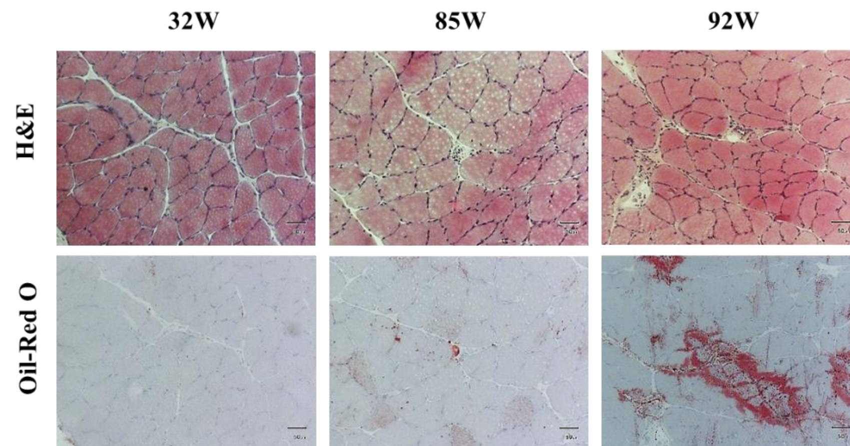

Fig 3. Age-related changes in H&E and Oil-Red O staining of soleus muscle of SD rats aged 32, 85, and 92 weeks. Magnification: ×100; Scale bar: 50 μm

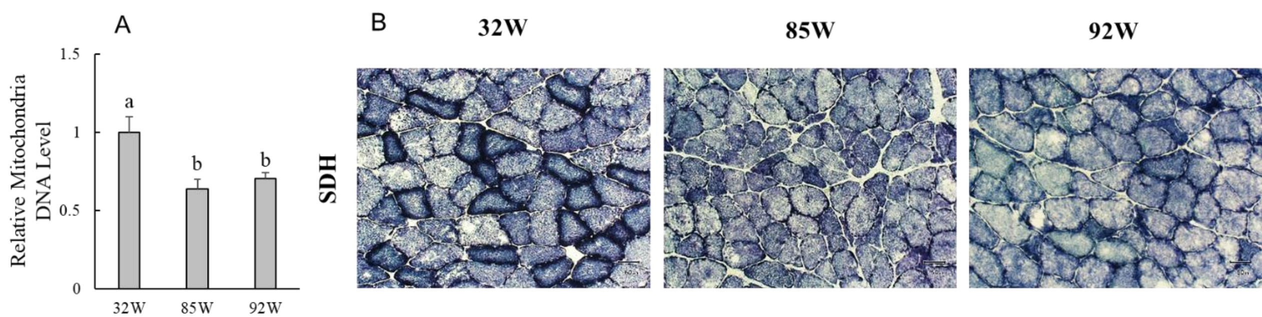

Fig 4. Age-related changes in mitochondrial function of soleus muscle.

(A) Relative mitochondrial DNA level in soleus muscle. The results were analyzed with one-way ANOVA followed by the Tukey-Kramer post-hoc test for multiple comparisons. The groups without the same letter present a statistically significant difference ($p<0.05$). The values shown represent mean ± SE of 3-6 rats. (B) SDH staining of soleus muscle section of SD rats aged 32, 85, and 92 weeks. Magnification: ×100; Scale bar: 50 μm

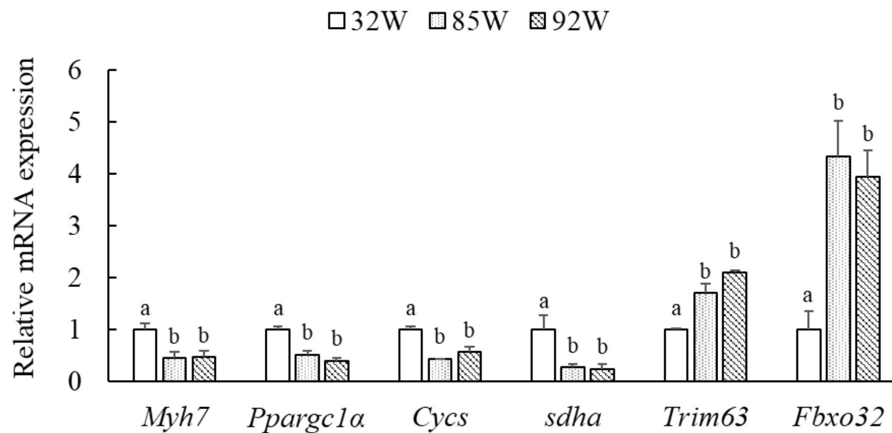

Fig 5. Age-related changes in relative mRNA expression levels of MyHC-I *(Myh7)*, PGC-1α (*Ppargc1α*), Cycs (*Cycs*), SDH (*sdha*), MuRF1 (*Trim63*), and Atrogin-1 (*Fbxo32*) in soleus muscle of SD rats aged 32, 85, and 92 weeks.

The values shown represent mean ± SE of 3-6 rats. The results were analyzed with one-way ANOVA followed by the Tukey-Kramer post-hoc test for multiple comparisons. The groups without the same letter present a statistically significant difference ($p<0.05$).

Age-related changes of* s*keletal muscle function

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 (Fig 4A). SDH staining of soleus muscle was performed to characterize mitochondrial enzyme function and myofiber oxidative capacity (19,20). 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 (Fig 4B). 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 (Fig 5). 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.

DISCUSSION

With the aging of rats, VO_2 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 (Fig 1A), suggesting that the aging process might contribute to a decline in O_2 consumption, which is indicated as a typical age-related change. Rivera also reported that VO_2max was lower in master runners than in young runners because of a diminished capacity to deliver and extract O_2 during exercise, in master runners [6]. 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 (Fig 1B). This indicates that the loss of spontaneous locomotor activity is an unavoidable phenomenon and a characteristic of the aging process. In a previous study by Conley et al, aged rats (24-27 months old) consistently presented lower spontaneous locomotor activity compared to young rats (3-6 months old), during the dark cycle (20:00-8:00) [33]. In another study, aged F344 rats showed 75-80% reduction in locomotor activity compared to young rats [42], which is also consistent with the result in this study.

Age-related muscle weakness and reduced aerobic capacity result not only in diminished physical performance, but also in metabolic disorders in humans [43, 44]. Reduced muscle mitochondrial function could contribute to age-related muscle dysfunction and reduced aerobic capacity [8]. In this study, it was observed that the relative mitochondrial DNA level was decreased significantly in the soleus muscle of rats aged 85 and 92 weeks compared to that of rats aged 32 weeks (Fig 4A). SDH activity is a performance for mitochondrial oxidative capacity [45, 46], and SDH staining results showed that mitochondrial oxidative capacity decreased in the soleus muscle of rats aged

85 and 92 weeks compared to that of rats aged 32 weeks (Fig 4B). Ermini et al. showed that SDH activity decreases in the muscles of old rats as compared to young rats [47]. Myosin heavy chain is an essential component of skeletal muscle and is associated with skeletal muscle contraction function [43, 48-49]. With advancing age, relative mRNA expression levels of PGC-1α, Cycs, and SDH decreased significantly in the soleus muscle of rats aged 85 and 92 weeks, compared to that of rats aged 32 weeks (Fig 5). PGC-1α plays a major role in metabolic regulation [34, 50], oxidative capacity [51], and regulation of respiration in skeletal muscle [52]. Dillon also showed that during aging, there is a decrease in PGC-1α expression in the heart and skeletal muscles [53]. Cycs is essential for energy production and mitochondrial respiration, as a component of the electron transport chain and as a mitochondrial marker protein [54, 55]. 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 [56]. In this study, the relative mRNA expression levels of MuRF1 and Atrogin-1 were 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 and skeletal muscle mass was decreased significantly in rats aged 85 and 92 weeks compared to that in rats aged 32 weeks (Table 2). Consistently, previous research by Honma et al. found that the weight of WAT (epididymal, mesenteric, and perirenal) was increased significantly with aging in SAMP10 mice [38]. In this study, 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 (Fig 3). Plasma FFA and cholesterol increased as the rats aged (Fig 2). Alessio found that usual age-related physiological changes include increased blood cholesterol, increased body fat, decreased lean body mass, and loss of bone density [57]. In another study, serum cholesterol levels in SD rats increased sharply in 24 months (173 ± 85 mg/dL), and the levels of serum cholesterol was tended to increase with age in primates [58]. 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.

Chapter III

Effects of long-term taurine supplementation on age-related changes in Sprague-Dawley rats and analysis of the molecular mechanisms underlying these effects

As an endogenous amino acid, taurine serves several physiological functions, the most primary being its role as an anti-fatigue molecule. Short-term taurine supplementation has the effect on exercise performance and duration. Administration of taurine (500 mg/kg BW) to mice after they had been running on a treadmill enhanced their recovery from endurance exercise-induced fatigue [23]. Administration of 3% taurine in drinking water for 1 month enhanced the exercise performance in rats, which correlated with the concentration of taurine in the muscle [24]. Oral administration of taurine (20, 100, and 500 mg/kg BW/day) to rats for 2 weeks improved exercise performance and duration, and maintained the concentration of taurine in the muscle [25]. Administration of taurine (500 mg/kg BW/day) to rats for 2 weeks maintained the levels of taurine in skeletal muscle during exercise and up-regulated physical endurance [26]. Taurine may be essential for skeletal muscle function and exercise capacity. Taurine deficiency in taurine transporter knockout (taut−/−) mice reduced the function of skeletal muscle [27]. Taurine depletion accelerates skeletal muscle aging and leads to early death in mice [28]. Taurine could improve the electrical and contractile properties of their muscle fibers [29]. It is reported by Miyazaki et al. that taurine affected skeletal muscle contraction and exercise performance by inhibiting oxidative stress, which alleviated both the increased levels of glutathione disulfide and the decreased concentration of taurine in the gastrocnemius (Gas) muscle, induced by exercise [25].

Given that taurine is associated with the function of skeletal muscle and exercise capacity as mentioned above, the effects of taurine on age-related changes are worth exploring. Numerous studies have been conducted on taurine administration in animal models. However, most of these have focused on induced animal taurine deficiency models (e.g., taurine transporter knockout, taurine antagonist, or dietary deficiency),

short-term taurine supplementation models, or supplementation of relatively high concentrations of taurine. Because taurine is commonly contained in various prepared beverages [16,59], we aimed to investigate the effects of a relatively low dose, long-term taurine administration on age-related changes in animal models. We hypothesized that long-term supplementation of taurine even at relatively lower concentrations may have improving effects on energy metabolism and skeletal muscle in aged rats via the function of AMP-activated protein kinase (AMPK). AMPK is a heterotrimeric protein kinase and has a function on the regulation of lipid metabolism [60], glucose uptake [61] in skeletal muscle, exercise performance [62, 63], and anti-aging [64]. Cheong and Chang reported that relatively lower concentration of taurine stimulates AMPK in L6 skeletal muscle cells [65]. Taurine may activate AMPK in skeletal muscle and have function on anti-aging effect through the function of AMPK. In this study, we examined the effects of long-term (5 months) administration of 0.5% (25 mg/kg/d) or 1% (50 mg/kg/d) taurine on changes in parameters, including VO_2 consumption, spontaneous locomotor activity, taurine levels in tissues and plasma, phosphorylated AMPK, and its related gene and proteins expressions in skeletal muscles, with age. The evidence of this study could be of potential clinical value for aging individuals.

MATERIALS AND METHODS

Materials

Taurine, NaOH, trichloroacetic acid, perchloric acid, acetonitrile, o-phthalaldehyde (OPA), sodium tetra borate, formalin, 2-mercaptoethanol, 1% eosin Y solution, glucose commercial kit, MgC_{l2}, sucrose, sodium fluoride, and α-tubulin antibody were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). KOH, ethanol, and nitro blue tetrazolium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rat L6 myoblasts (JCRB9081) were purchased from JCRB Cell Bank (Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and 0.02% ethylenediaminetetraacetic acid (EDTA) were purchased from MP Biomedicals (Santa Ana, CA, USA); penicillin, streptomycin, and 0.25% trypsin were from Invitrogen (Carlsbad, CA, USA). Hematoxylin from Merck KGaA (Darmstadt, Germany), and Mount-quick and Mount-quick aqueous from DAIDO SANGYO (Tokyo, Japan). Sepasol-RNA I Super G and sodium succinate were purchased from Nacalai Tesque (Kyoto, Japan). RNase inhibitor, ReverTra Ace® qPCR Master Mix, and gDNA remover kit were purchased from Toyobo Co., Ltd. (Osaka, Japan). KAPA SYBR® FAST qPCR kit was purchased from Kapa Biosystems (Wilmington, MA, USA). Antibodies against AMP-activated protein kinase α (AMPKα), phosphorylated AMPKα, and glucose transporter type 4 (GLUT4) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against PGC-1α, myocyte enhancer factor 2A (MEF2A), and myoglobin were from Santa Cruz Biotechnology (CA, USA).

Animal experiments

All animal experiments were conducted in accordance with the guidelines of

Okayama Prefectural University and the laws and notifications of the Japanese government. All animal experiments were approved by the Animal Care and Use Committee of Okayama Prefectural University (protocol number 31-5). Male SD rats were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were housed individually in an air-conditioned room at 25 °C with an alternating 12 h light/dark cycle (light, 8:00–20:00). All the rats were allowed free access to commercial food (CE-2, CLEA Japan, Inc., Tokyo, Japan) and water. Food intake and body weight were measured daily. Respiratory metabolism was measured with an O_2/CO_2 metabolism measuring system (model MK-5000; Muromachi Kikai, Tokyo, Japan), and spontaneous locomotor activity was measured using an infrared sensor (PYS-001; Muromachi Kikai). Each rat was kept in a sealed chamber for 24 h. The airflow was maintained at 3.0 L/min and the temperature was 25 °C; the animals had free access to water and food.

SD rats aged 32 weeks were assigned randomly to one of the following treatment groups: water (control), 0.5% taurine (w/v), or 1% taurine (w/v). The rats were fed a normal laboratory diet for 2 weeks for acclimatization, and were administered their respective doses from age 34 weeks, 5 d a week (5 mL/kg BW, n = 3–5/group), until 56 weeks of age. The administered dose of taurine was 25 mg/kg body weight for the 0.5% taurine group and 50 mg/kg body weight for the 1% taurine group. Blood samples were collected at ages 33 (before administration), 37, 41, 45, 49, 53, and 56 weeks in heparinized tubes and centrifuged at 3,000 × g and 4 °C for 10 min and plasma was obtained. Respiratory metabolism and spontaneous locomotor activity were measured at 33, 37, 41, 45, 49, and 53 weeks. At 56 weeks of age, the rats were anesthetized by intraperitoneal pentobarbital sodium injection (100 µL/100 g BW), and tissue samples were collected 24 h after the final treatment administration. Portions of the tissues were

frozen in liquid nitrogen and stored at -80 °C until the subsequent measurements. Other tissues were isolated, cramped, and freeze-dried (TOKYO RIKAKIKAI, Tokyo, Japan) for the measurement of taurine levels.

Culture of L6 cells

L6 myoblasts were grown to confluence in DMEM supplemented with 10% (v/v) FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C containing 5% CO_2. Once myoblasts reached 80% confluence, differentiation of myotubes was induced by culturing them in a differentiation medium [DMEM containing 2% (v/v) horse serum]. Myotubes were harvested 11 d after differentiation, and experimental procedures were initiated.

Biochemical analysis

Freeze-dried tissue samples were homogenized with 2 mL of 0.5 N perchloric acid and centrifuged at 362 × g and 4 °C for 10 min (Pierno et al. 1998). The supernatant was neutralized with 5N and 1N of KOH, centrifuged at 362 × g at 4 °C for 10 min, and collected in a separate tube. Trichloroacetic acid 10% (v/v) was added to the precipitate the plasma proteins. The suspension of plasma samples was shaken for 1 h and centrifuged at 815 × g at 4 °C for 15 min and the supernatant was neutralized with 5N and 1N of KOH and collected. L6 myotubes were treated with 300 μM taurine for 60, 120, 180, and 240 min, and the medium was collected. The pretreatment of the medium was the same as for plasma. Taurine was derivatized with OPA derivatizing reagent [200 μL 25 mg/mL OPA in ethanol, 10 μL 2-mercaptoethanol, and 2.5 mL 0.1 M sodium tetraborate buffer (adjusted to pH 9.5 with NaOH)] and measured as a fluorescent adduct on an HPLC system (Waterfield 1994; Terrlink et al. 1994; Terrill et al. 2015). The HPLC

system (Shimadzu Corp., Tokyo, Japan) was equipped with LC-20AB HPLC pumps, a CTO-20A column oven, an SPD-M20A detector, and a reverse-phase column (Shim Pack VP-ODS separation, 250 L × 4.6; Shimadzu Corp., Tokyo, Japan). Phosphate buffer mixed with acetonitrile (70:30) was used as the mobile phase. The flow rate was 1 mL/min, the injection volume was 5 µL, the wavelength for detection was 338 nm, and the column temperature was 40 °C. Taurine was derivatized by mixing it with an equal volume of the OPA derivatizing reagent for 1 min before injecting the reaction mixture into the column (Ferreira et al. 1997). Plasma glucose level was measured using a commercial kit. SDH activity was measured by spectrophotometer (U-2900, Hitachi High-Tech Corp., Tokyo, Japan) (Spinazzi et al. 2012; Maruta and Yamashita 2020). Gas muscle was homogenized and added into reaction solution (25 mM potassium phosphate buffer (pH 7.5), 1 mg/ml bovine serum albumin, 300 µM potassium cyanide, 20 mM succinate and 80 µM DCPIP). The reaction mixture was incubated at 37˚C for 8 min, and plotted the baseline at 600nm for 2 min. The reaction was started by adding 50 µM DUB and was measured at 600 nm for 3 min.

Histological analysis

Gastrocnemius (Gas) muscle tissue was cut into 10-µm sections using a Leica CM3050 S cryostat (Leica Microsystems, Wetzlar, Germany) at -20 °C and fixed in 10% (v/v) formalin (Wako Pure Chemical Industries Ltd., Osaka, Japan). The sections were stained with hematoxylin and eosin (H&E) and mounted with Mount-Quick mounting medium. For SDH staining, Gas sections were air-dried at 20–25 °C for 5 min and incubated in a solution consisting of 50 mM phosphate buffer, 50 mM sodium succinate, and 0.5 mg/mL nitro blue tetrazolium at 37 °C for 40 min (Mangum et al. 2016). The

sections were washed in dH2O for 3 min and mounted with Mount-Quick aqueous mounting medium. Images were captured with a CCD camera (Olympus Optical, Tokyo, Japan) at ×100 magnification.

qRT-PCR analysis

Total RNA was isolated from frozen tissue samples with Sepasol-RNA I Super G. RNase inhibitor was added according to the manufacturer's instructions. Total RNA was quantified, and cDNA was prepared using the ReverTra Ace® qPCR Master Mix and gDNA remover kit. Quantitative RT-PCR was performed on a StepOnePlus detection system (Applied Biosystems, Foster City, CA, USA) using the KAPA SYBR® FAST qPCR Master Mix Kit to determine the content of specific mRNAs. Data were normalized to β-actin mRNA, and the expression levels were compared to those for the water control group. The qRT-PCR primers used were as follows: rat β-actin (actb) forward: 5′-GGAGATTACTGCCCTGGCTCCTA-3′, reverse: 5′-GACTCATGTACTCCTGCTTGCTG-3′; rat PGC-1α (Ppargc1α) forward: 5′-GACCCCAGAGTCACCAAATGA-3′, reverse: 5′-GGCCTGCAGTTCCAGAGAGT-3′; rat SDH forward: 5′-TGGGGCGACTCGTGGCTTTC-3′, reverse: 5′-ATCTCCAGTTGTCCTCTTCCA-3′; rat cytochrome c (Cycs) forward: 5′-ACCGGGACGTCTCCCTAAGA′, reverse: 5′-CTTCCGCCCAAACAGACCA-3′; rat MEF2A (Mef2a) forward: 5′-ATGAGAGGAACCGACAGGTG-3′, reverse: 5′-TATCCGAGTTCGTCCTGCTT-3′; rat GLUT4 (Slc2a4) forward: 5′-CTCATGGGCCTAGCCAATG-3′, reverse: 5′-GGGCGATTTCTCCCACATAC-3′.

Western blot analysis

Tissue samples were homogenized in extraction buffer [25 mM Tris HCl [pH 8.0], 1 mM EDTA, 0.5 mM dithiothreitol, 10 mM $MgCl_2$, 0.25 mM sucrose, 50 mM sodium fluoride, and 1% (w/v) protease inhibitor] and centrifuged at 1,109 × g at 4 °C for 10 min. The protein content in the supernatant was determined using the Bradford assay. Aliquots containing ~30 μg protein were subjected to 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to an Immobilon-P membrane (Merck KGaA, Darmstadt, Germany). After blocking with 3% BSA/TBST, the membranes were incubated with primary antibodies overnight at 4 °C, washed thrice with TBST (Tris-buffered saline with Tween-20), and incubated with HRP-conjugated secondary antibodies for 60 min. For highly sensitive detection, the membranes were incubated with the primary antibodies, followed by biotin-conjugated secondary antibodies for 30 min, and then HRP-conjugated streptavidin for 15 min. The membranes were washed three times with TBST. The chemiluminescence reaction was performed for 5 min with ImmunoStar LD (Wako Pure Chemical Industries) according to the manufacturer's protocol. The chemiluminescent signals were visualized and quantified with ImageQuant LAS-4000 (Fujifilm, Tokyo, Japan).

Statistical analysis

Data are presented as means ± standard error (SE). Treatment means were subjected to one-way ANOVA followed by the Tukey–Kramer post-hoc test (Statcel 2, OMS Inc., Tokorozawa, Japan) or Dunnett's multiple comparisons test (Ystat 2013, Igakushoin, Tokyo, Japan) (* $p < 0.05$, ** $p < 0.01$ relative to the water control).

RESULTS

Effect of taurine on body weight gain, respiratory metabolism, and spontaneous locomotor activity

To examine the effect of taurine supplementation at relatively low dose on respiratory metabolism and spontaneous activity during aging, VO_2 and spontaneous locomotor activity were measured for SD rats from 33 to 53 weeks of age. 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 (Fig 1A). In the resting period, VO_2 of the water group significantly decreased from age 45 weeks, whereas that of the 0.5% taurine group only decreased from 53 weeks, and that of the 1% taurine group did not decrease significantly as compared to rats aged 33 weeks. 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 (Fig 1B). With increasing age, the spontaneous locomotor activity in the water group during the resting period decreased significantly at the age of 53 weeks, whereas in the taurine groups, no significant change was observed. The body weight gain increased steadily as rats aging; there was no significant difference in the water and taurine groups from 34 to 55 weeks of age (Fig 1C).

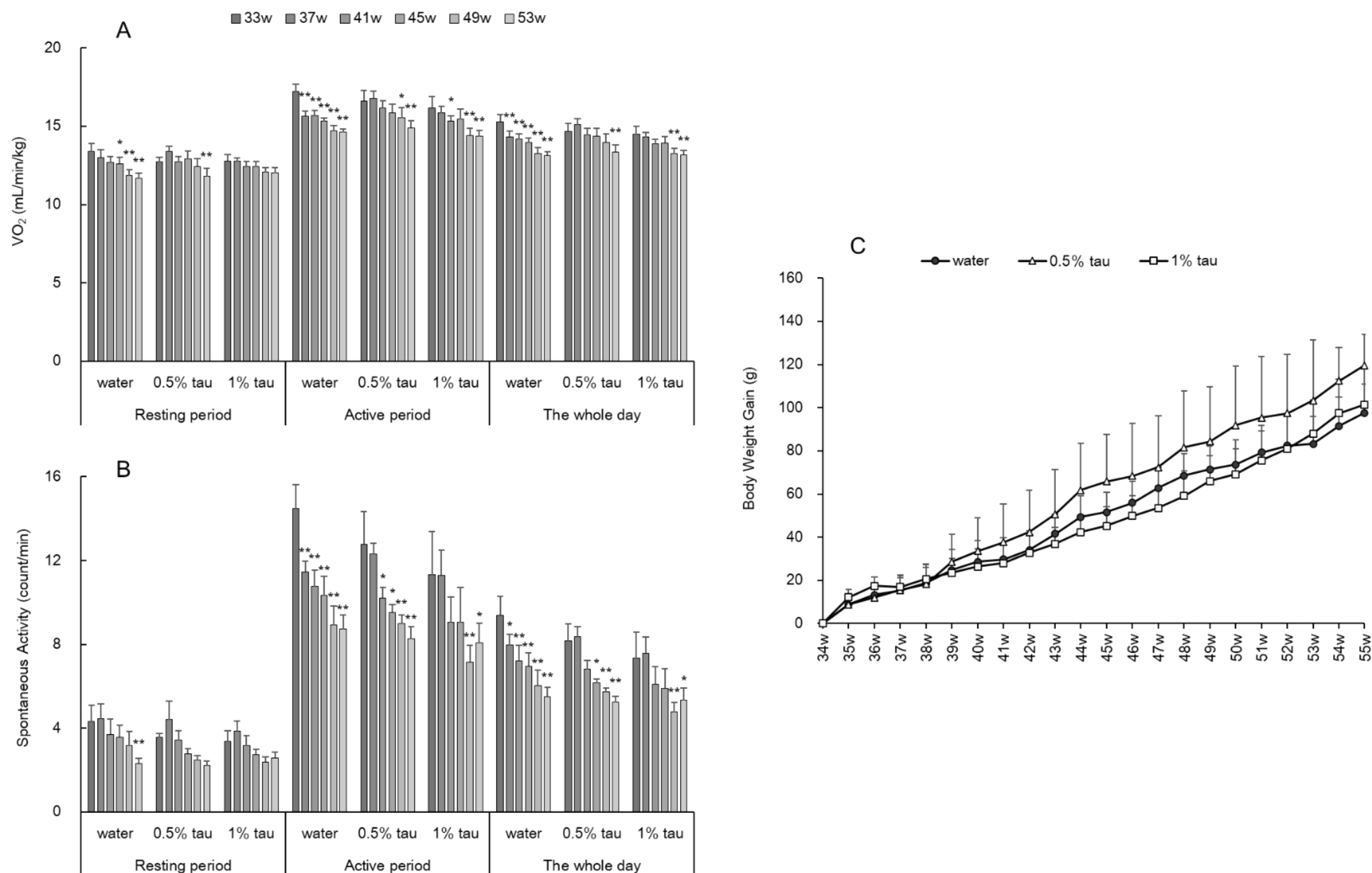

Fig 1. VO2 in SD rats from 33 to 53 weeks of age in the resting, active, and whole day periods (a). Spontaneous locomotor activity of SD rats from 33 to 53 weeks of age in the resting, active, and whole day periods (b). Average body weight gain of SD rats from 34 to 55 weeks of age (c). Values shown represent means ± SE for 5 rats. *p <0.05, **p <0.01 statistically significant versus the value obtained at 33 weeks of age. Results were analyzed using Dunnett's test

Taurine levels in plasma and tissues, taurine incorporation into L6 myotube cells, and the effect of taurine on plasma glucose level

Taurine levels in plasma and tissues containing skeletal muscles were measured to examine the effect of long-term of low dose taurine supplementation on the incorporation of taurine to tissues. Plasma taurine levels (μM) in the 0.5% and 1% taurine groups were higher from age 37 to 53 weeks compared with that in the water group (Fig 2A).

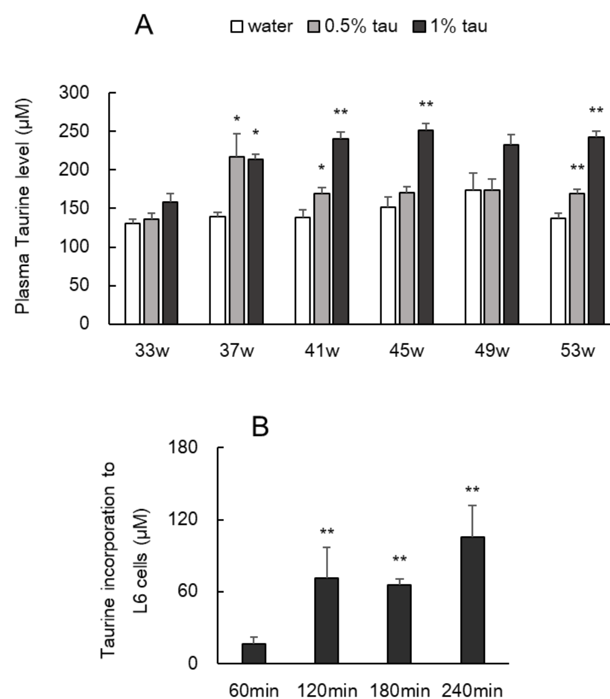

Fig 2. Plasma taurine level (μM) in SD rats from 33 to 53 weeks of age in the water, 0.5% taurine, and 1% taurine groups (a). Values shown represent means ± SE for 5 rats. *p <0.05, **p <0.01 statistically significant versus the value obtained in the water group. Taurine incorporation (μM) into L6 cells, 60, 120, 180, and 240 min after treatment with 300 μM taurine (b). Values shown represent means ± SE for 4–5. *p <0.05, **p <0.01 statistically significant versus the values after 60 min in the treatment group. Results were analyzed using Dunnett's test

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 (Fig 2B). 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 (Fig 3).

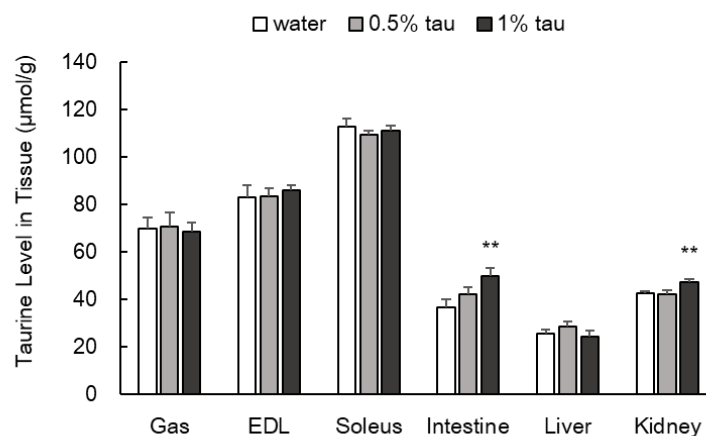

Fig 3. Taurine content (µmol/g) in gastrocnemius (Gas) muscle, extensor digitorum longus (EDL) muscle, soleus muscle, intestine, liver, and kidney of Sprague-Dawley (SD) rats at 56 weeks of age in the water, 0.5% taurine, and 1% taurine groups in 24 hr after the taurine administration. Values shown represent means ± SE for 5 rats. *p <0.05, **p <0.01 statistically significant versus the values in the water group. Results were analyzed using Dunnett's test

There were no changes in taurine levels in skeletal muscles and liver among water and taurine groups. To examine the glucose lowering effect of taurine during aging process, glucose level was measured. The level of glucose (mg/dL) in the plasma was significantly lower in the 0.5% and 1% taurine groups than in the water group at 56 weeks of age (Fig 4).

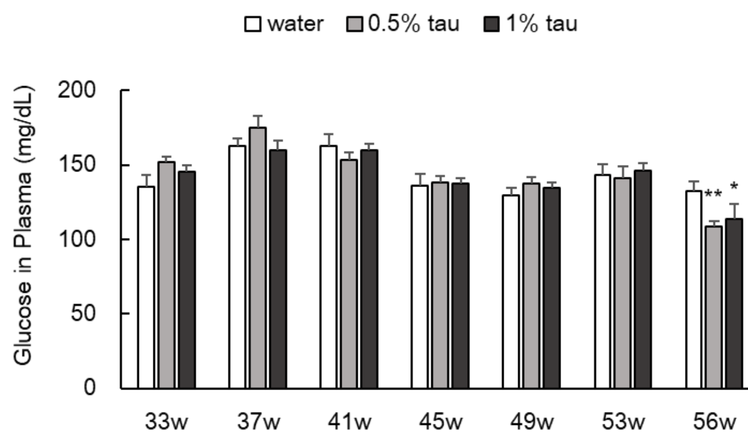

Fig 4. Plasma glucose levels in SD rats at 33 to 56 weeks of age in the water, 0.5% taurine, and 1% taurine groups. Values shown represent means ± SE for 5 rats. *p <0.05, **p <0.01 statistically significant versus the value in the water group. Results were analyzed using Dunnett's test

Effect of taurine on phosphorylated AMPK and genes and proteins related with skeletal muscle function

To examine the effect 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 (Fig 5).

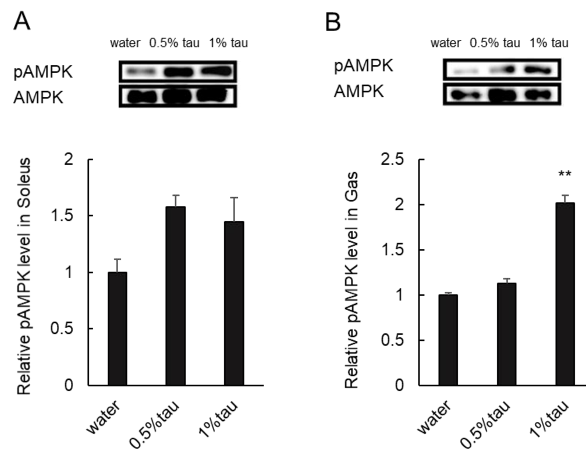

Fig 5. Effect of taurine administration on the phosphorylation of AMPK in soleus and Gas muscles of SD rats. Values shown represent means ± SE for 3–5 rats. **p <0.01 statistically significant versus the values in the water group. Results were analyzed using Dunnett's test

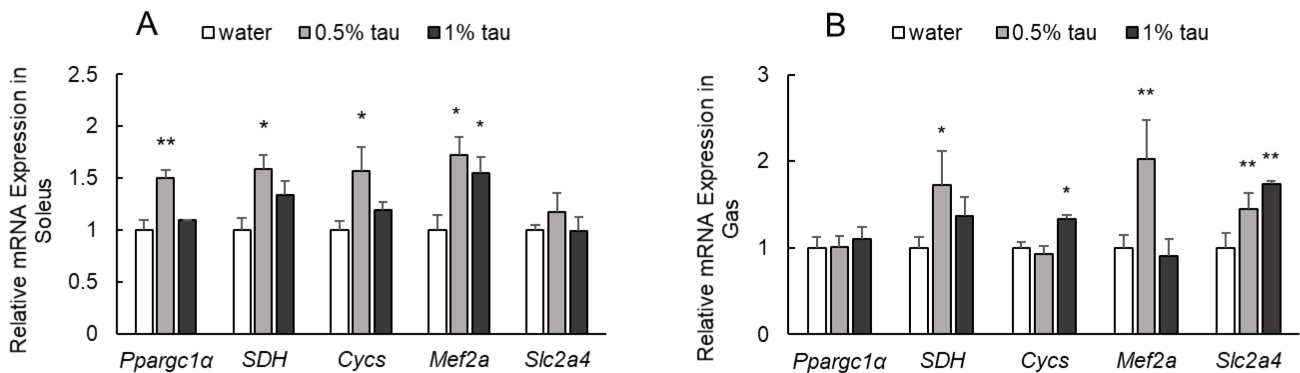

Fig 6. Effect of taurine administration on relative mRNA expression of Ppargc1α, SDH, Cycs, Mef2a, and Slc2a4 in the soleus muscle of SD rats at 56 weeks of age (a). Effect of taurine administration on relative mRNA expression of Ppargc1α, SDH, Cycs, Mef2a, and Slc2a4 in Gas muscle of SD rats at 56 weeks of age (b). Values shown represent means ± SE for 3–5 rats. *p <0.05, **p <0.01 statistically significant versus the values in the water group. Results were analyzed using Dunnett's test

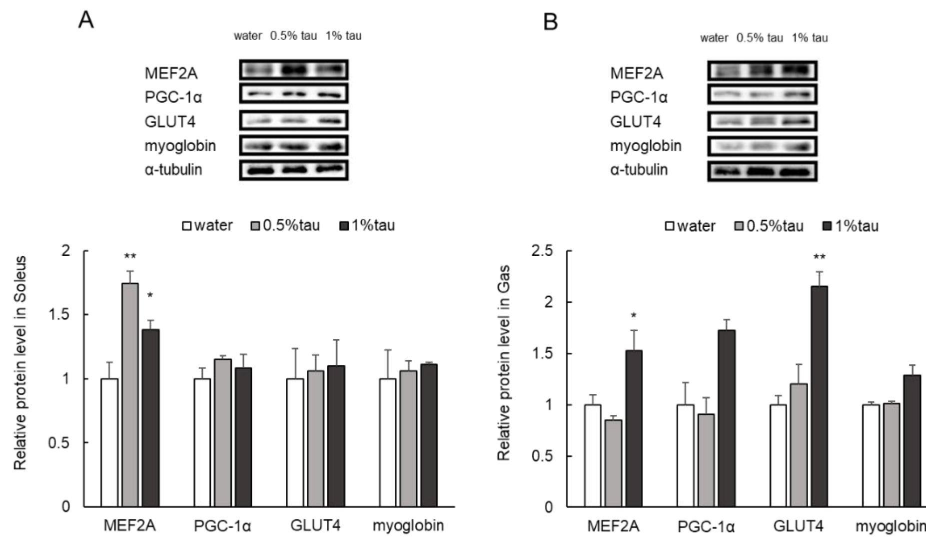

Fig 7. Effect of taurine administration on relative levels of MEF2A, PGC-1α, GLUT4, and myoglobin in the soleus muscle of SD rats(a). Effect of taurine administration on the relative levels of MEF2A, PGC-1α, GLUT4, and myoglobin in the Gas muscle of SD rats (b). Values shown represent means ± SE for 3–5 rats. *p <0.05, **p <0.01 statistically significant versus the values in the water group. Results were analyzed using Dunnett's test

Expression levels of *Ppargc1α*, *SDH*, *Cycs*, and *Mef2a* mRNAs in the soleus muscle were significantly increased in the 0.5% taurine group compared to that in the water group (Fig 6A). The expression of *Mef2a* mRNA was also significantly upregulated in the 1% taurine group relative to that in the water group. In Gas muscle, mRNA expression levels of *SDH*, *Mef2a*, and *Slc2a4* were significantly increased in the 0.5% taurine group. The expression levels of *Cycs* and *Slc2a4* mRNAs were significantly enhanced in the 1% taurine group and both taurine groups, respectively, compared with that in the water group (Fig 6B). Protein level of MEF2A was significantly increased in the soleus muscle in both

taurine groups (Fig 7A) and 1% taurine group in the Gas muscle (Fig 7B). The level of GLUT4 protein in Gas muscle was increased significantly in the 1% taurine group and tended to be increased in 0.5% taurine group.

Histological analysis

The SDH staining was performed to characterize the mitochondrial enzyme function and the oxidative capacity of myofibers (Mangum et al. 2016; White et al. 2011). Fig. 8 shows H&E and SDH staining and SDH activity of Gas muscle. Gas muscle of rats expressed higher in SDH protein by taurine supplementation and showed significantly higher activity in both taurine groups than those of water group.

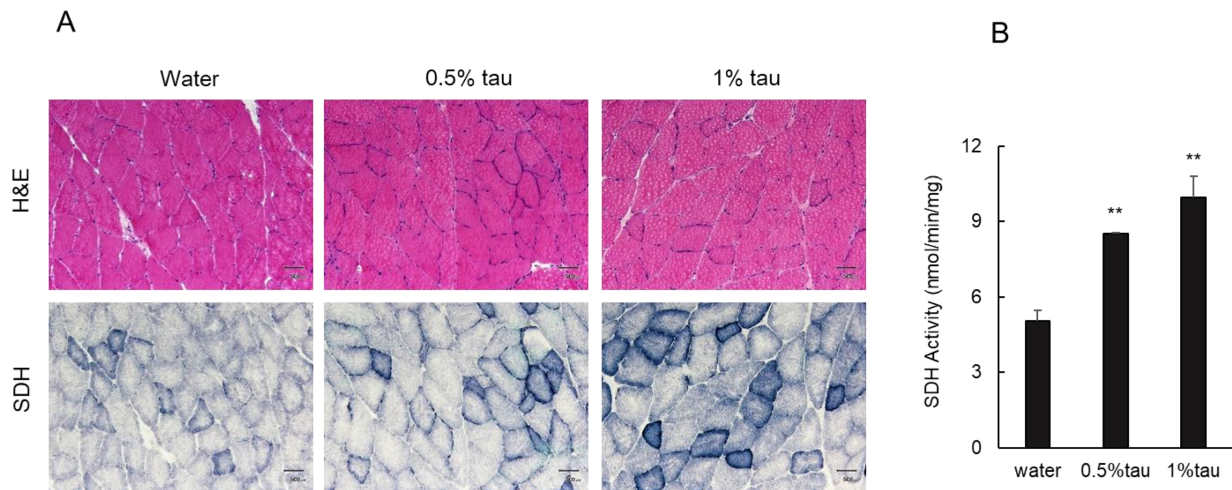

Fig 8. Representative H&E and SDH staining of Gas muscle sections from SD rats in the water, 0.5% taurine, and 1% taurine groups at 56 weeks of age (a). Magnification: ×100. Scale bar, 500 μm. SDH activity in Gas muscle of SD rats in the water, 0.5% taurine, and 1% taurine groups at 56 weeks of age (b).

DISCUSSION

As shown in Fig. 1A, VO_2 in the water group during the active and whole day periods decreased significantly every month as the rats aging, which was consistent with the result in Chapter II. In contrast, VO_2 in the 0.5% and 1% taurine groups did not decrease significantly until 49 or 53 weeks of age. 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, as shown in Fig. 1B, during the active and whole day periods, the spontaneous locomotor activity of rats in the water group decreased significantly every month, however, in the 0.5% taurine group, significant decrease was observed 1 or 2 months later than in the water group, and it did not decrease significantly in the 1% taurine group until 49 weeks of age. 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 (Fig 2A). There was no significant change in taurine level among groups in each skeletal muscle after the long-term administration of taurine. Sved et al. reported the concentration of taurine in plasma and tissues after dosing in rats receiving 1, 7, or 14 days of taurine at 30 or 300 mg/kg/day [66]. They describe that overall the concentrations in the different tissues containing muscle did not show major differences between controls and treated animals, and were not greatly influenced by dose level or duration of treatment. They discuss that exogenous taurine rapidly equilibrates with endogenous body pools and that any excess is rapidly eliminated by the kidneys. However, 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. Taurine

supplementation of 1% stimulated phosphorylation of AMPK significantly and 0.5% taurine supplementation tended to stimulate the phosphorylation in Gas muscle (Fig 5). AMPK plays a key role in the regulation of energy metabolism [54, 60, 67], oxidative capacity [68], exercise capacity [62, 63], and aging processes [64]. AMPK also has a role in stimulation of glucose uptake in skeletal muscle [61] 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 via its inhibiting action for HDAC4/5, which is transcriptional repressor of MEF2 proteins [6, 69]. Moreover, AMPK has an inducing effect on the expression of MEF2A [70, 71]. 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 (Fig 4). And the expressions of *GLUT4* gene and protein were significantly induced in the Gas muscle of rats in the 1% taurine group (Fig 6B and 7B). In 0.5% taurine group, it shows a significant increase of GLUT4 gene and an increase tendency of GLUT4 protein in Gas muscle (Fig 6B and 7B). Furthermore, the expression of MFE2A protein was significantly increased in 1% taurine group in Gas muscle (Fig 7B). Taurine group of 0.5% shows significant increases in *MEF2A* gene and protein in Gas muscle (Fig 6B and 7B). 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. Other studies show that taurine supplementation could normalize blood glucose and plasma insulin levels in CHT male C57BL/6J mice [72], or glucose supplementation following the administration of taurine significantly decreased the blood glucose levels in male guinea pigs [73].

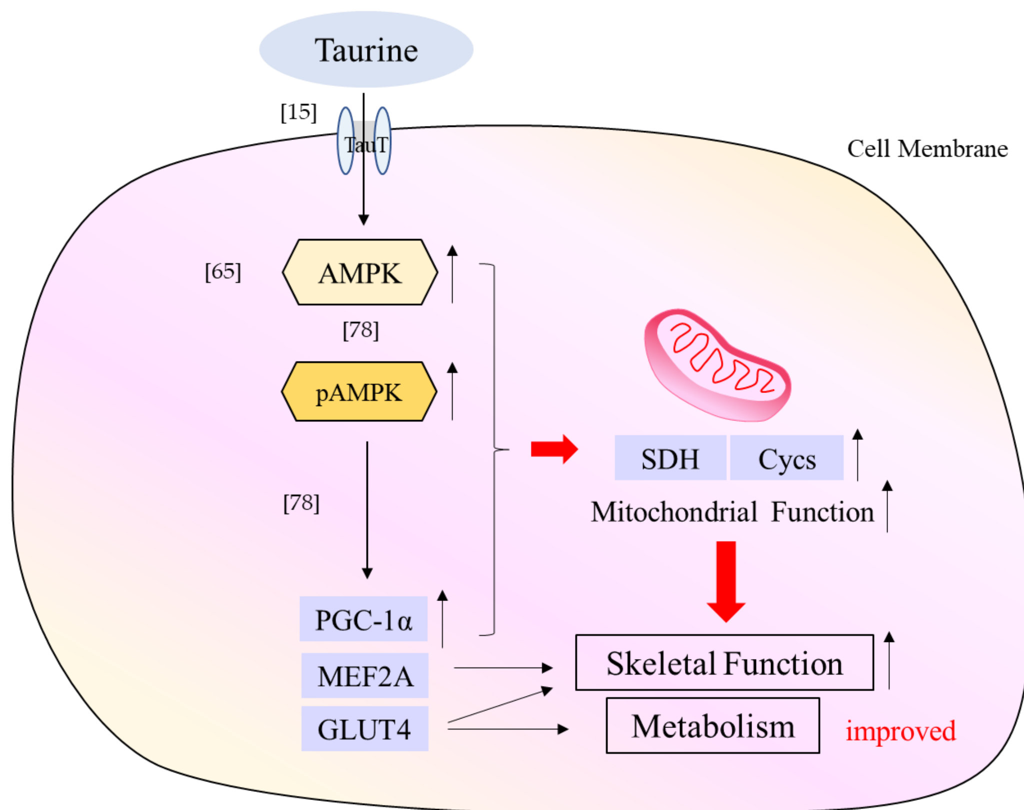

Fig 9. Analysis of the molecular mechanisms of taurine effect in skeletal muscle

Aging is associated with dysregulation of energy metabolism and mitochondrial dysfunction. SDH activity is a performance of mitochondrial oxidative capacity [45, 46]. Comparison between young and old rats showed that SDH activity decreases in the muscles of old rats [47]. *SDH* gene expression in the soleus and Gas muscles of taurine groups tended to be higher than that in the water group (Fig 6). SDH activity in Gas muscle also result significantly higher in both taurine groups (Fig. 8). Cytochrome *c* is essential for energy production and mitochondrial respiration, as a component of the electron transport chain and mitochondrial markers [54, 55]. 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 compared to that in the water 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 [74-76], and it is mutually activated with MEF2A [77]. In this study, the expression of PGC-1α protein tended to be increased in Gas muscle of 1% taurine group (Fig 7B). *Cytochrome c* and *GLUT4* genes were increased significantly in 1% taurine group (Fig 6B). And SDH activity was also increased in taurine groups (Fig 8). AMPK may increase the expression of PGC-1α in the muscle by 1% taurine supplementation, lead to increased *MEF2A* gene as well as elevate the levels of *cytochrome c*, *SDH*, and *GLUT4* genes, which are a subset of genes regulated by PGC-1α [50, 78]. 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. The potential mechanisms of taurine effect in skeletal muscle is shown in Fig 9. In this study, taurine supplementation might phosphorylate and activate AMPK, and increased mitochondria function by SDH and Cycs, which might be mediated by PGC-1α. In addition, taurine supplementation increased MEF2A and GLUT4 mediated by AMPK, and elevate skeletal muscle function. This may be the potential mechanism of taurine anti-aging effects on skeletal muscle.

Chapter IV Conclusion Remarks

Aging process might contribute to a decline in the O_2 consumption, spontaneous locomotor activity, and glucose oxidation of rats aged 90 weeks compared to that of rats aged 32 weeks. With advancing age, skeletal muscle mass decreased significantly in rats aged 85 weeks, and 92 weeks compared to that in rats aged 32 weeks. With aging, the levels of genes *Myh7*, *Ppargc1α*, *Cycs*, and *sdha*, and mitochondrial DNA, which are related to skeletal muscle function and muscle oxidative capacity, decreased significantly in the soleus muscle of rats aged 85 and 92 weeks compared to that of rats aged 32 weeks. In addition, lipid accumulation in skeletal muscle and weight of white adipose tissue (WAT) around kidney increased with age in rats.

Long-term administration of taurine could attenuate the age-related decline in O_2 consumption and spontaneous locomotor activity. Taurine might modulate age-related changes in respiratory metabolism and skeletal muscle function via PGC-1α, SDH, Cycs, MEF2A, glucose transporter 4 (GLUT4), and myoglobin, which are regulated by the activation of AMP-activated protein kinase (AMPK). 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.

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