Experimental Gerontology 98 (2017) 120–123

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short report

Flavan 3-ol delays the progression of disuse atrophy induced by hindlimb suspension in mice

Mao Ito [a], Naoto Kudo [a], Yuji Miyake [a], Tatsuya Imai [a], Tomoki Unno [b], Yoko Yamashita [c], Yoshihisa Hirota [a], Hitoshi Ashida [c], Naomi Osakabe [a,*]

[a] Department of Bio-science and Engineering, Shibaura Institute of Technology, Japan
[b] Department of Health and Nutrition, Tokyo Kasei Gakuin University, Japan
[c] Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Japan

ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form 23 June 2017
Accepted 12 July 2017
Available online 12 August 2017

Section Editor: Holly M. Brown-Borg

Keywords:
Hindlimb suspension
Flavan 3-ols
Ubiquitin ligase
p70S6 kinase

ABSTRACT

Periods of skeletal muscle disuse, for example due to a sedentary lifestyle or bed rest, are associated with aging and can lead to muscle atrophy. We previously found that the flavan 3-ol fraction derived from cocoa (FL) enhanced energy expenditure with metabolic changes in skeletal muscle. In the present study, we examined the effect of FL on disuse muscle atrophy induced by hindlimb suspension in mice. Male C57BL/6J mice were assigned to four groups as follows: unsuspended-vehicle, unsuspended-FL, suspended-vehicle, and suspended-FL. Mice in the vehicle treatment groups were administered distilled water and those in the FL treatment groups were dosed with FL (50 mg/kg/day) for 2 weeks. The weights of the gastrocnemius (GC), tibialis anterior (TA), and soleus (SOL), but not the extensor digitorum longus (EDL), decreased significantly in mice with hindlimb suspension (−11.8%, −16.5%, and −41.0%, respectively). This reduction in GC, TA, and SOL mass was inhibited by FL (−5.3%, +2.0%, and −16.6%, respectively). The FL increased the EDL weight >20% with or without hindlimb suspension. The protein level of the ubiquitin ligase, muscle ring finger-1, in the SOL was significantly increased by hindlimb suspension, but inhibited by treatment with FL. Protein expression of p70S6 kinase in the SOL was significantly decreased by hindlimb suspension, and FL treatment inhibited this change. These results suggested that FL delayed disuse muscle atrophy by metabolic alteration.

1. Introduction

Skeletal muscle atrophy is associated with greater morbidity and mortality and reduced independence, especially in older populations (Rudrappa et al., 2016). A period of skeletal muscle disuse is related with aging, often due to a sedentary lifestyle or bed rest, and can lead to muscle atrophy (Magne et al., 2013), impaired contractile performance, and overall muscle weakness. Preventing disuse muscle atrophy requires a mechanistic understanding of the cellular signaling pathways that regulate both protein synthesis and breakdown in muscle (Powers, 2014). The hindlimb suspension disuse model leads to significant skeletal muscle atrophy, particularly in the soleus (SOL). This model provides information on the morphological and molecular changes responsible for the mechanisms of disuse-induced muscle loss (Brooks and Myburgh, 2014). A recent report suggested that muscle loss by disuse was induced to a similar extent in young vs. older animals, but apoptotic DNA fragmentation was significantly increased in the young animals (Siu et al., 2005)

It is well established that cocoa is rich in flavan 3-ol monomers and oligomers, such as catechins and B-type procyanidins that are linked by C4–C8 bonds (Hammerstone et al., 1999). In addition, numerous randomized, controlled trials have confirmed that cocoa improves metabolic disorders (Tokede et al., 2011). In a previous study, we confirmed that after a single dose of FL there were metabolic changes in skeletal muscle, such as increases in peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) expression, phosphorylation of AMP kinase in the gastrocnemius (GC) (Matsumura et al., 2014), with significant plasma adrenaline rising. After repeated ingestion of FL, there was an increase in the mitochondria copy number in the GC and SOL thought to be caused by PGC-1α up-regulation (Watanabe et al., 2014), It has been reported that over expression of PGC-1α reduced disuse atrophy and slow-to-fast myofiber type transition (Wang et al., 2017). In addition, we confirmed that the biochemical changes in skeletal muscle described above, induced by a single dose of FL, were completely reduced by

Abbreviations: EDL, Extensor digitorum longus; FL, Flavan 3-ol; GC, Gastrocnemius; MuRF1, Muscle ring finger-1; p70S6K, p70S6 kinase; PGC-1α, Proliferator-activated receptor gamma coactivator-1 alpha; PI3K, Phosphoinositide 3-kinase; SOL, Soleus; TA, Tibialis anterior.

* Corresponding author at: Department of Bio-science and Engineering, Shibaura Institute of Technology, 307 Fukasaku, Munumaku, Saitama 337-8570, Japan.
E-mail address: nao-osa@sic.shibaura-it.ac (N. Osakabe).

http://dx.doi.org/10.1016/j.exger.2017.07.010
0531-5565/

pretreatment with a β-adrenaline receptor blocker (Kamio et al., 2016). This result suggested the activity was adrenomimetic. The β2 adrenaline agonists, such as formoterol (Ryall et al., 2007) and clenbuterol (Sirvent et al., 2014), induce skeletal muscle growth and hypertrophy by down-regulating specific proteolytic systems; in addition, β-adrenergic stimulation induces up-regulation of PGC1-α and subsequent transcriptional changes (Frier et al., 2012). These growth-promoting properties of the β2-adrenergic agonists caused increased muscle mass and reduced muscle atrophy from muscular dystrophy, denervation, and hindlimb suspension in animal models (Dutt et al., 2015). The β2-adrenergic agonists inhibit ubiquitin ligases, such as atrogin-1/MAFbx and muscle ring finger-1 (MuRF), and also increase p70S6 kinase (p70S6K), a downstream target of phosphoinositide 3-kinase (PI3K)/Akt/FOXO in animal atrophy models (Goncalves et al., 2012; Ryall and Lynch, 2008.) In addition, recent reports suggest that disuse atrophy is inhibited by the supplementation of polyphenols, epigallocatechin gallate (Meador et al., 2015), quercetin (Le et al., 2014), and resveratrol (Momken et al., 2011). These observations suggested FL has the potential to prevent disuse muscle atrophy. In the present study, we examined the effect of repeated oral administration of FL on disuse muscle atrophy induced by hindlimb suspension in mice.

2. Materials and methods

Thirty-two mice were fed a basal diet for 7 days and divided randomly into four groups: unsuspended-vehicle treatment, unsuspended-FL treatment, suspended-vehicle treatment, and suspended-FL treatment. Mice in the vehicle treatment groups were administered distilled water orally; while, animals in the FL treatment groups were dosed with FL (gavage administration, 50 mg/kg bw/day) for 14 days. At the end of this treatment period, the animals were sacrificed under anesthesia, and the GC, TA, EDL, and SOL were removed. The MuRF1 and p70S6K levels in the SOL were determined by western blotting. Further details on the materials, methods, and statistical analysis are described in the Supplementary Material.

3. Results

The body weight, mean food intake, total food intake, measured and relative weight (mg and mg/kg body weight) of the liver, heart, kidney, adrenal gland, spleen, epididymis adipose, perirenal adipose, brown adipose, and subcutaneous adipose of the experimental animals are shown in Table 1 and S-Table 1 in the Supplementary Material. There were no significant differences between the experimental groups in food consumption, body weight, or cardinal tissue weights. The percent changes were calculated using the relative weight of the hindlimb skeletal muscles such as the GC, TA, SOL, and EDL and are shown in Table 1 and Fig. 1. The relative weights of the GC, TA, and SOL in the suspended-vehicle treatment group were significantly decreased compared with the unsuspended-vehicle treatment (−11.8%, −16.5%, and −41.0%, respectively, $p < 0.01$). Repeated administration of 50 mg/kg FL prevented these changes, and the relative weights are in the GC (−5.3%), TA (+2.0%), and SOL (−16.6%). In contrast, FL administration increased the EDL relative mass significantly with or without hindlimb suspension (Table 1 and Fig. 1). Compared with the unsuspended-vehicle treatment group, the EDL relative mass in the unsuspended-FL treatment group and suspended-FL treatment group increased 20.5% and 20.0%, respectively ($p < 0.01$).

The levels of the ubiquitin ligase, MuRF1, in the SOL are shown in Fig. 2a. Compared with the unsuspended-vehicle group, MuRF1 increased 56% in the suspended-vehicle group ($p < 0.01$, Fig. 2a). Administration of FL did not influence the levels of MuRF1 in the SOL in unsuspended animals, but did reduce the elevation of MuRF1 in the treated suspension group to only a 27.2% increase compared with the unsuspended-vehicle group. The SOL p70S6K level was significantly reduced in the hindlimb suspension-vehicle group (46.2%, compared with the unsuspended-vehicle group, $p < 0.01$), as shown in Fig. 2b. Treatment with FL showed a trend to increase in p70S6K of unsuspended animals. The reduction of the SOL p70S6K level induced by hindlimb suspension was reversed significantly by FL treatment (150% compared with the suspended-vehicle group, $p < 0.05$) (Fig. 2b).

4. Discussion

It has been reported that an interim hindlimb suspension period causes significant muscle atrophy in mammals (Brooks and Myburgh, 2014), similar to immobilization with aging. In the present study, we found significant muscle atrophy in the GC, TA, and SOL after 14 days of hindlimb suspension; however, this atrophy was not observed in the EDL (Fig. 1). Rodent muscles that express predominantly slow motor units appear to be more sensitive to the unloading stimuli than muscles expressing primarily fast motor units (Sung et al., 2013).

In addition, repeated FL administration prevented the decrease of muscle mass in the GC, TA, and SOL (Table 1 and Fig. 1). In the EDL, the relative muscle mass was significantly increased by ingestion of FL, as shown Table 1 and Fig. 1.

Numerous studies have shown that β2-agonists are powerful anabolic agents that trigger skeletal muscle hypertrophy (Lynch and Ryall, 2008). The β2-agonists can attenuate and/or reverse the decrease in skeletal muscle mass in rodent disuse atrophy models, as well as human patients (Kissel et al., 2001). It was reported that β2-agonist administration activates the PI3K-Akt/mTOR pathway (Kline et al., 2007). The Akt activates the downstream kinase mTOR, which stimulates p70S6K, ultimately culminating in enhanced protein synthesis (Berdeaux and Stewart, 2012). In present the study, hindlimb suspension induced p70S6K down regulation in the SOL, as shown in Fig. 2b. In contrast, repeated FL inhibited this change, and

Table 1
Body weight, food intake, and hindlimb skeletal muscle weight after repeated oral administration of vehicle or FL with or without hindlimb suspension.

	Unsuspended		Suspended	
	Vehicle	FL	Vehicle	FL
Body weight (initial, g)	27.3 ± 1.9	27.0 ± 1.8	27.0 ± 0.8	26.5 ± 1.0
Body weight (final g)	26.3 ± 1.5	25.8 ± 3.3	25.8 ± 2.1	24.3 ± 1.3
Mean food intake (g/day)	3.7 ± 0.3	3.6 ± 0.3	3.7 ± 0.3	3.4 ± 0.2
Total food intake (g)	51.3 ± 4.1	50.3 ± 4.1	52.0 ± 4.2	47.5 ± 3.1
Gastrocnemius (mg)	337.8 ± 16.4	321.4 ± 23.4	273.6 ± 21.4**	291.4 ± 10.2*
Gastrocnemius (mg/kg)	12,891.3 ± 764.6	12,560.2 ± 937.5	10,707.4 ± 1468.3	12,045.7 ± 838.9
Tibialis anterior (mg)	104.1 ± 7.3	105.8 ± 11.4	91.8 ± 4.2*	98.0 ± 7.7
Tibialis anterior (mg/kg)	3975.7 ± 392.9	120.1 ± 265.5	3576.6 ± 207.6	4039.9 ± 257.0
Soleus (mg)	20.5 ± 1.6	19.2 ± 3.4	11.9 ± 2.0**	14.0 ± 1.3**
Soleus (mg/kg)	780.7 ± 54.4	748.3 ± 117.6	461.2 ± 53.3	376.5 ± 31.5
Extensor digitorum longus (mg)	22.5 ± 4.8	28.0 ± 2.8**	22.2 ± 0.8	26.3 ± 3.5*
Extensor digitorum longus (mg/kg)	855.9 ± 162.2	1088.9 ± 49.7	864.0 ± 50.9	1079.2 ± 89.4

Each value represents the mean and standard deviation ($n = 8$, each). Significantly different from the unsuspended vehicle group: *$p < 0.05$, **$p < 0.01$.