Role of dietary flavonoids in oxidative stress and prevention of muscle atrophy

Rie Mukai and Junji Terao*

Department of Food Science, Graduate School of Nutrition and Biosciences, University of Tokushima, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan

Received: July 12, 2013 / Accepted: August 6, 2013

Abstract Functional foods for the prevention of disuse muscle atrophy (DMA) are expected to improve the quality of life (QoL) of bedridden people. Ubiquitin ligases targeting muscle protein degradation, atrogin-1 and muscle-specific ring finger protein (MuRF-1), are critical in the degradation of muscle protein, and oxidative stress induced by mitochondrial dysfunction seems to be involved in muscle atrophy. Dietary antioxidants that attenuate the oxidative stress in skeletal muscle are strong candidates as food ingredients for preventing DMA. The antioxidative flavonoid quercetin was found to prevent DMA by attenuating the induction of atrogin-1/MuRF-1 in mice undertaking the tail suspension test. Several studies revealed that dietary quercetin accumulates in skeletal muscle after metabolic conjugation during absorption. There are many arguments that antioxidant activity is essential for dietary flavonoids to exert their preventive effects, but modulation of the IGF-1 signaling pathway is definitively involved in the mechanism of prevention. Nevertheless, dietary flavonoids (including quercetin) may be potential food factors in the prevention of muscle atrophy. Dietary flavonoids are expected to prevent DMA by attenuating oxidative stress derived from mitochondrial dysfunction.

Keywords: flavonoid, antioxidant, oxidative stress, disuse muscle atrophy, ubiquitin ligase

Introduction

“Oxidative stress” denotes the imbalance between the generation of reactive oxygen species (ROS) and their elimination in cellular and extracellular systems or the entire body of the organism. In a narrow sense, ROS involve the superoxide anion radical (O$_2^•$−), hydroxyl radical (•OH), hydrogen peroxide (H$_2$O$_2$), and singlet molecular oxygen (‘O$_2$). O$_2^•$− can generate reactive peroxynitrite (ONOO−) by reacting with nitric oxide, and the perhydroxyl (or hydroperoxy) radical (•OOH) under acidic conditions. Fig. 1 illustrates the relationship between the successive generation of ROS with their related active species and antioxidant defense (which eliminates these species for the prevention of dysfunction of body systems). In 1969, McCord and Fridovich$^1$ first discovered the enzyme superoxide dismutase (SOD), which catalyzes the dismutation of O$_2^•$− into an oxygen molecule and H$_2$O$_2$. It is now understood that the resulting H$_2$O$_2$ is converted to water by glutathione peroxidase (GPx). H$_2$O$_2$ escapes from the generation of highly reactive •OH, which is readily generated by one-electron transfer from transition metal ions (the Fenton reaction). Therefore, two antioxidative enzymes, superoxide dismutase (SOD) and GPx, cooperate in the detoxification of O$_2^•$− and subsequent reactive species in the body$^2$.

Recently, the role of peroxiredoxins (a ubiquitous family of antioxidant enzymes) in relation to the cellular elimination of H$_2$O$_2$ has attracted much attention$^3$. In any case, oxygen radicals generated secondarily from O$_2^•$− (i.e., •OH, ONOO− and •OOH) cannot be eliminated by these antioxidative enzymes. Also, O$_2^•$−-scavenging enzymes have not been found in any biological system.

The human body provides small-molecule antioxidants as a system for the elimination of •OH, ONOO− , •OOH and ’O$_2$ as well as other reactive species such as lipid peroxyl radicals. Vitamin C and vitamin E are recognized as powerful hydrophilic and lipophilic antioxidants of dietary origin, respectively. They can scavenge oxygen radicals by acting as electron and/or hydrogen donors. Dietary carotenoids seem to be effective specifically for the quenching of ’O$_2$.$^4$ In addition, flavonoids are assumed to be involved in dietary antioxidants, and exert physiological functions in the body through their antioxidant activity.$^5$ Flavonoids are plant polyphenols containing a diphenylpropane structure. The reducing property of the phenolic hydroxyl group is usually responsible for their activity in scavenging oxygen radicals.

Oxidative stress is thought to cause the initiation and/or promotion of degenerative diseases such as atherosclerosis, cancer, and disorders of the central nervous system. Therefore, the dietary intake of flavonoids may be help-
ful in human health by participating in the antioxidant network together with vitamin E, vitamin C and other biological antioxidants in the human body. Interestingly, recent studies have suggested that dietary antioxidants (including flavonoids) also upregulate the expression of antioxidant enzymes by promoting the Nrf-2 signal transduction pathway. However, overconsumption of dietary antioxidants may induce harmful effects in the human body. Hence, antioxidants could exert dual effects in the body. Therefore, knowledge of the bioavailability of dietary flavonoids and the molecular mechanisms of action underlying their physiological and pharmacological functions should be obtained for application in the prevention and treatment of disuse muscle atrophy (DMA).

This review article focuses on the role of dietary flavonoids in oxidative stress and the prevention of DMA. Oxidative stress is closely related to DMA, and this muscle dysfunction causes severe problems in aging societies in which there are increasing numbers of bedridden people.

Oxidative stress and DMA

ROS and muscular-protein degradation. Muscle atrophy, which is the major determinant of dysfunction of disuse muscle, is caused by an imbalance between protein synthesis and its degradation. DMA is triggered by immobilization, lack of physical activity, and weightlessness. Oxidative stress is known to accompany the proteolytic pathways (ubiquitin–proteasome, lysosomal, and calpain pathways) that induce DMA. It has been reported that two ubiquitin ligases, atrogin-1 and muscle-specific ring finger protein 1 (MNF-1), are critical for the development of DMA, and expression of these genes is regulated by Forkhead box-O transcription factors (FOXOs) signaling pathways. ROS induce these ubiquitin-ligases in myotube cells or primary cultured-muscular cells via the nuclear factor-kappa B (NF-kB) pathway and/or FOXOs pathway. Calpain has an important role in H2O2-induced myotube atrophy. Myosin degradation by calpain is accelerated under the oxidative stress induced by the Fenton reaction with ferrous ion and H2O2.

Oxidative stress in DMA. Mitochondrial disorders have been suggested to be responsible for ROS generation in atrophied skeletal muscles. Therefore, mitochondria-targeted antioxidants may protect skeletal muscle against immobilization-induced muscle atrophy. Unloading, immobilization, or denervation are typical experimental models to induce DMA in animals and humans. Immobilization enhances the generation of ROS (including •OH) in atrophied skeletal muscle. Nitric oxide also accelerates DMA in accordance with activation of the FOXO signaling pathway under immobilization or unloading conditions. In one animal study, levels of markers of oxidative stress in atrophied muscle were elevated. That is, 8-hydroxy-2′-deoxyguanosine (8-OHdG), an index of oxidative damage in DNA, accumulated in the gastrocnemius muscle during unloading. Proteins conjugated with 4-hydroxy-2-nonenal (4-HNE), a secondary product of lipid peroxidation, and primary lipid hydroperoxides (LOOHs) were found in atrophied muscle. Levels of non-esterified fatty acid hydroperoxides
NEFA-OOHs were significantly elevated in the mitochondria isolated from atrophied muscle\(^{(18)}\). NEFA-OOHs (but not their hydroxyl derivatives) induced mitochondrial dysfunction with increasing oxidative stress in the mitochondrial matrix of skeletal muscle, in which complex I was assumed to be the major site of \( \text{O}_2^- \) production\(^{27}\). Hindlimb unloading-induced protein oxidation resulted in the accumulation of protein carbonyls in the myofibril of skeletal muscle\(^{(28)}\). Taken together, it can be concluded that oxidative stress derived from ROS generation in mitochondria induces DMA by modulating the signaling pathway of protein degradation involving the ubiquitin–proteasome system (Fig. 2).

**Physiological conditions related to oxidative stress in DMA.** It seems that antioxidant defense operates by sensing the enhancement of oxidative stress during DMA. For example, the reduced form of glutathione (GSH) can act as an antioxidant in a state of oxidative stress and results in the oxidized form of glutathione (GSSG). Immobilization has been shown to induce the conversion of GSH to GSSG in rat muscles\(^{(20,29)}\). Resistance against DMA can be attenuated by the apoptosis of cells in skeletal muscle if the rat is depleted of GSH\(^{(20)}\). Levels of GPx and catalase (which remove \( \text{H}_2\text{O}_2 \)) were significantly reduced in the soleus of rats after hindlimb unloading\(^{(22)}\). In a human intervention study, heme oxygenase-1 (HO-1), which is recognized as an antioxidative enzyme induced by oxidative stress through the Nrf-2 signal transduction pathway, was enhanced significantly\(^{(10)}\). Manganese (Mn)-type SOD is localized in mitochondria, and Mn deficiency seems to be responsible for the oxidative stress related to mitochondrial dysfunction. Interestingly, the concentration of Mn was increased in the mitochondrial fraction of rat atrophied muscle\(^{(29)}\). Skeletal muscle-specific Mn-SOD-deficient mice showed increases in oxidative damage and loss of enzymatic activity in the mitochondrial respiratory chain complex\(^{(31)}\). Hindlimb unloading resulted in a small decrease in Mn-SOD activity in the rat soleus muscle\(^{(22)}\).

Ions of copper and iron have also been suggested to contribute to oxidative stress. The concentration of iron ions in atrophied muscle was increased, but the level of copper ions was stable during the development of muscle atrophy\(^{(29)}\). The iron concentration in the microsomal fraction continued to increase throughout DMA. In addition, the level of thiobarbituric acid reactive substances (TBARS), a biomarker of lipid peroxidation, and iron content of the muscle were elevated during immobilization in rats\(^{(32)}\). An iron-chelator, deferoxamine, suppressed the increase in levels of TBARS in atrophied rat muscle\(^{(32)}\). It is, therefore, likely that iron ions participate in oxidative stress by inducing lipid peroxidation in the microsomal fraction under DMA.

**Bioavailability and antioxidant ability of dietary flavonoids.**

**Intestinal absorption and bioavailability of flavonoids.**

The bioavailability of flavonoids is dependent upon the structure of each flavonoid, and varies considerably with coexisting food ingredients. Manach et al.\(^{(33)}\) compared the bioavailability of different classes of flavonoids by

---

\( \text{Fig. 2} \) Relationship between oxidative stress induced by mitochondrial dysfunction and disuse muscle atrophy.
converting the published data to a dose of 50 mg, assuming that the bioavailability is increased relative to intake in a linear manner. They found that bioavailability was decreased in the order of isoflavones > flavan-3-ols > flavanones > flavonols.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a typical flavonol-type flavonoid present in its glycoside form in plant foods. A study in Japanese women showed that the mean daily intake of flavonoids is 16.7 mg/day and that of quercetin is 9.3 mg/day. Onions are a major source of dietary quercetin, which is present as O-glucosides. The bioavailability parameters of quercetin O-glucosides have been estimated to be 1.46 ± 0.45 μM for peak plasma concentration (C_{max}) and 2.5 ± 1.2% for urinary excretion. After ingestion, the first step in the absorption of these flavonoid glucosides is deglycosylation, which occurs in the lumen of the small intestine (Fig. 3). Flavonoid O-glucosides are hydrolyzed by cytosolic β-glycosidase (CBG) or lactase-phlorizin hydrolase (LPH). Cleavage of flavonoid aglycones from sugar moiety is necessary for efficient absorption from the small intestine. Without cleavage in the small intestine, flavonoid glycosides reach the lower digestive tract, where enterobacteria can hydrolyze glycosides and decompose the basic phenylpropane structure.

Finally, flavonoids are converted to a wide range of phenolic acids. The free aglycones already present in the food (or those released from glycosides with the involvement of intestinal enzymes and enterobacteria) can pass into enterocytes via passive transport.

In intestinal epithelial cells, free flavones and flavonoids are immediately subjected to the action of phase-II enzymes to form glucuronide, sulfate and/or methylated metabolites. The resulting conjugated metabolites enter the circulatory system and are transported via the portal vein to the liver, where the metabolites are subjected to further reactions from phase-II enzymes. Flavonoid metabolites in the liver are partly transported to bile and returned into the gastrointestinal tract through enterohepatic circulation. However, some conjugated metabolites are transported to the entire body via circulation, and are finally excreted into the urine. Fig. 3 illustrates the fate of dietary quercetin glucosides after oral intake.

Studies in rats and pigs revealed the tissue distribution of quercetin after long-term feeding of this flavonol. Quercetin and its conjugated metabolites were distributed in various tissues (including skeletal muscle). Table 1 shows the summary data for the accumulation of dietary flavonoids in the skeletal muscle of rodents after long-term or short-term intake. Interestingly, quercetin aglycone was found in several tissues, but most of the quercetin could have been derived from artificial deconjugation during extraction.

**Antioxidant activity of flavonoids and their metabolites.**

Bors et al. claimed that three partial structures contributed to the radical-scavenging activity of flavonoids: (1) a 2,3-double bond in conjugation with the 4-oxo group, which is necessary for delocalization of an unpaired electron from the B-ring; (2) a 2,3-double bond in conjugation with the 4-oxo group, which is necessary for delocalization of an unpaired electron from the B-ring; and (3) hydroxyl groups at the 3- and 5-positions, which are necessary for enhancement of radical-scavenging activity (Fig. 4). Quercetin has been suggested to exert powerful radical-scavenging activity among flavonoids because this flavonol possesses all the partial structures contributing to its radical scavenging activity. Its metabolic conversion during absorption enables the attenuation or loss of antioxidant activity because the catechol group frequently disappears due to O-methylation and/or glucuronidation/sulfation of the hydroxyl group at the B-ring. However, one of the major quercetin metabolites, quercetin 3-O-glucuronide (Q3GA), still possesses considerable antioxidant activity because its catechol group is unchanged.

**Antioxidants and DMA**

**Effect of antioxidants on muscular oxidative stress.** Dietary antioxidants seem to suppress DMA. For example, supplementation of vitamin E suppressed the TBARS level and expression of ubiquitin-ligases, but elevated the GSH level in rat skeletal muscle. Vitamin E was found to decrease the level of TBARS in the breast muscle of chickens. Supplementation with cysteine to the diet normalized the ratio of GSH to GSSG in rat skeletal muscle, and suppressed reduction of muscle mass and fragmentation of myosin heavy chain by inhibiting the ubiquitin–proteasome system. An antioxidative polyphenol, resveratrol, suppressed unloading-induced decreases in muscle mass and levels of TBARS and H2O2. Conversely, polyphenols, obtained from extracts of grape seeds and dried tomatoes, suppressed lipid peroxidation in the breast muscle of chickens.

**Antioxidative and anti-atrophic effects of quercetin and related flavonoids in skeletal muscle.** It has been reported that quercetin injected into the gastrocnemius muscle prevents DMA with a reduction of oxidative stress in unloading mice. Quercetin also suppressed the expression of MuRF-1 and atrogin-1 in the gastrocnemius muscle. Short-term feeding of quercetin elevated the expression of genes associated with mitochondrial biogenesis and mitochondrial DNA in the soleus muscle. Catechins (dietary antioxidants distributed in tea and chocolate) also suppressed the expression of ubiquitin ligase. Tea catechins involving epigallocatechin gallate (EGCG) enhanced the antioxidative potential and suppressed protein carbonyls in the myofibrils of rat atrophied muscle, which was induced by unloading. EGCG prevented mitochondrial loss in the skeletal muscle of diabetic Goto-Kakizaki rats. Intake of tea catechins was effective for suppressing the decline in physical performance and the...
Table 1. Flavonoid accumulation in muscle

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Animal type</th>
<th>Intake condition</th>
<th>Feeding period</th>
<th>Type of muscle</th>
<th>Concentration (total of aglycons and metabolites) (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>Fisher 344 rat</td>
<td>0.1% quercetin diet</td>
<td>11 wks</td>
<td>Quadriceps</td>
<td>1.10 (^{(57)})</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Fisher 344 rat</td>
<td>1% quercetin diet</td>
<td>11 wks</td>
<td>Quadriceps</td>
<td>4.16 (^{(57)})</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Pig</td>
<td>25 mg/kg body weight</td>
<td>Single dose</td>
<td>Longissimus dorsi</td>
<td>0.14 (^{(57)})</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Pig</td>
<td>50 mg/kg body weight</td>
<td>4 wks</td>
<td>Longissimus dorsi</td>
<td>0.10 (^{(57)})</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Pig</td>
<td>50 mg/kg body weight</td>
<td>4 wks</td>
<td>Diaphragm</td>
<td>0.14 (^{(57)})</td>
</tr>
<tr>
<td>Naringenin</td>
<td>C57BL/6</td>
<td>0.17% naringenin diet</td>
<td>22 days</td>
<td>Gastrocnemius</td>
<td>0.56 (^{(57)})</td>
</tr>
<tr>
<td>8-PN</td>
<td>C57BL/6</td>
<td>0.2% 8-PN diet</td>
<td>22 days</td>
<td>Gastrocnemius</td>
<td>4.12 (^{(57)})</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>Wistar rat</td>
<td>0.2% hesperetin diet</td>
<td>4 weeks</td>
<td>Gastrocnemius</td>
<td>0.71 (^{(57)})</td>
</tr>
<tr>
<td>Chalcones</td>
<td>ICR mice</td>
<td>200 mg ashitaba extract/kg body weight</td>
<td>Single dose</td>
<td>No information</td>
<td>4-hydroxyderricin: 0.04 xanthoangenol: 0.005 (^{(57)})</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Lactating ewe</td>
<td>50 % red clover silage contained diet (red clover containing isoflavones)</td>
<td>One month</td>
<td>No information</td>
<td>less than 10 nmol/g (^{(57)})</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>C57BL/6</td>
<td>0.5% bilberry diet</td>
<td>2 wks</td>
<td>No information</td>
<td>Below the limit of detection (10 pmol/g tissue) (^{(57)})</td>
</tr>
</tbody>
</table>

Fig. 3 Transportation pathway of dietary flavonoids after oral intake.
The mechanism of DMA was suggested to involve mitochondrial dysfunction leading to ROS release. This action induces oxidative stress within the cell, and there is considerable evidence that oxidative stress is closely related to the promotion of protein gradation such as the ubiquitin ligase–proteasome system. Alternatively, the oxidative stress causes damage to cellular and extracellular components. Finally, the function of skeletal muscle declines gradually. Flavonoids are recognized as dietary antioxidants, and it was recently found that they accumulate in target muscles to carry out their antioxidant ability after metabolic conversion to conjugated derivatives upon absorption in the intestine and liver. Although the precise mechanism of action is unclear, several studies have demonstrated that flavonoids are helpful in the normal function of skeletal muscle. In particular, quercetin is present in a wide variety of vegetables, and is a promising flavonoid for the prevention of DMA. Human intervention studies should be carried out concerning the practical application of flavonoids for bedridden people.

**Conclusion**

The mechanism of DMA was suggested to involve mitochondrial dysfunction leading to ROS release. This action induces oxidative stress within the cell, and there is considerable evidence that oxidative stress is closely related to the promotion of protein gradation such as the ubiquitin ligase–proteasome system. Alternatively, the oxidative stress causes damage to cellular and extracellular components. Finally, the function of skeletal muscle declines gradually. Flavonoids are recognized as dietary antioxidants, and it was recently found that they accumulate in target muscles to carry out their antioxidant ability after metabolic conversion to conjugated derivatives upon absorption in the intestine and liver. Although the precise mechanism of action is unclear, several studies have demonstrated that flavonoids are helpful in the normal function of skeletal muscle. In particular, quercetin is present in a wide variety of vegetables, and is a promising flavonoid for the prevention of DMA. Human intervention studies should be carried out concerning the practical application of flavonoids for bedridden people.

**Acknowledgments**

This work was supported by JSPS KAKENHI (grant numbers 23780136 and 22380077) and by the Program for Promotion of Basic and Applied Research Innovations in Bio-oriented Industry in Japan.

**References**

38: 1543-1552.


between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 130: 2243-2250.


