Ginkgo biloba Extract EGb 761 and Wisconsin Ginseng Delay Sarcopenia in Caenorhabditis elegans

Zhiming Cao,1 YanJue Wu,1 Kenneth Curry,3 Zhixin Wu,3 Yves Christen,4 and Yuan Luo1,2

1Department of Pharmaceutical Sciences, School of Pharmacy, and
2Center for Integrative Medicine, School of Medicine, University of Maryland, Baltimore.
3Department of Biological Sciences, University of Southern Mississippi, Hattiesburg.
4IPSEN, Paris, France.

Previously we reported that the standardized Ginkgo biloba extract EGb 761 extended life span and increased stress resistance in Caenorhabditis elegans. In this study, pharmacological modulation of age-dependent muscle degeneration, or sarcopenia, was determined. Transgenic C. elegans strain (PD4251) expressing green fluorescent protein (GFP)-MYO-3, localized in body wall muscles and vulval muscle nuclei, were fed with EGb 761 or Wisconsin Ginseng, and muscle integrity was analyzed by quantification of GFP fluorescence. Both EGb 761 and Wisconsin Ginseng significantly delayed sarcopenia. Ginseng was more effective in worms of more advanced age, which is consistent with the ultrastructural changes observed by transmission electron microscopy. Furthermore, both agents ameliorated age-associated decline of locomotive behaviors including locomotion, body bend, and pharyngeal pumping. These results suggest that pharmacological extension of life span is a consequence of maintaining functional capacity of the tissue, and that C. elegans is a valid model system for testing therapeutic intervention for delaying the progress of sarcopenia.

Sarcopenia refers to the progressive loss of skeletal muscle mass and strength in senescence (1), a condition that accompanies the natural aging process. In humans, skeletal muscles undergo a gradual 3%–8% decline in muscle mass per decade after the age of 30. The pace and extent of sarcopenia proceeds faster in populations older than 60 years (6%–15%), resulting in approximately 40% of skeletal muscle fibers in persons older than 80 years (2–4). Despite the well-accepted fact that muscle fiber atrophy and muscle fiber loss, as well as a decrease in the cross-sectional area of the remaining muscle fibers, are strongly correlated with sarcopenia (2,5–7), the underlying mechanisms involved in its progression still remain unresolved.

A significant reduction in muscle function parallels the muscle mass decline involved in sarcopenia, leading to impaired physiological function and physical performance in older adults. Although sarcopenia is not directly lethal to affected individuals, it can lead to the development of a number of clinical consequences, which include decreased mobility, increased risks of falling and fractures, reduced energy intake, and impaired thermoregulatory and respiratory function and insulin resistance (8–12). The severity of sarcopenia could prevent older individuals from living independently and have an adverse impact on the quality of life of the geriatric population. Due to the increasing elderly population, sarcopenia has been recognized as one of the marked problems in the developed world because of its health care consequences and its socioeconomic implications (13,14). Age-related muscle deterioration not only occurs in the skeletal muscle of human and other mammalian species, but also in invertebrates. It has been demonstrated that sarcopenia accompanies the age-dependent functional decline in the nematode Caenorhabditis elegans, similar to those longer-lived vertebrates (15,16).

Caenorhabditis elegans is a well-studied and widely used model organism for many genetic and biomedical investigations. In the past decade, this species has become a valuable genetic system for the study of aging (17) and for identifying targets for possible pharmacological modulation of life spans (18). Herndon and colleagues (15) explored the major cellular changes of a number of important cell types in aging worms. They revealed that the nervous system in senescent nematodes stays largely intact, although the muscles experience a gradual and progressive deterioration, i.e., sarcopenia. As in humans, sarcopenia compromises mobility in the worms and serves as a marker for increased mortality (15). Consistent with this view, dissociated muscle integrity was shown to correlate with age-related functional deficits (19) and could be reduced by treatment of the worms with muscarinic agonist (20) or natural antioxidants (21).

Taken together, findings obtained from human and worm studies strongly suggest that sarcopenia represents an important biomarker of aging and can serve as an independent predictor for morbidity and mortality in population-based studies (22). Sarcopenia is a complex process involving many molecular, cellular, and functional alterations. However, the underlying mechanisms responsible for these deleterious changes remain unclear. The similarity of worm muscle degeneration to sarcopenia in humans raises the possibility of using C. elegans to explore candidate factors contributing to muscle aging and could therefore facilitate...
the elucidation of sarcopenia mechanisms in humans. Based on the intimate link between aging and sarcopenia, it is hypothesized that drug interventions aimed at slowing senescence and extending life expectancy might render some protection against age-dependent muscle deterioration.

Increasing experimental evidence supports the life-span extension and delaying of aging by pharmacological agents in *C. elegans* (23–26), including Egb 761 reported from our laboratory (27). Egb 761 is a standardized extract from the leaves of *Ginkgo biloba*. In addition to its many clinical uses for dementia (28), Egb 761 has been shown to enhance cognition, stress resistance, and longevity in mammals (29–33). American ginseng is another commonly used herbal medicine in the United States. It is derived from the root of the ginseng plant and is known to improve physical performance and health maintenance and to have immunostimulating, anticancer, and neuroprotective activities (34,35). American ginseng cultivated in Wisconsin (WG) and Illinois exhibits a different ginsenoside profile (36,37).

The original goal of this study was to examine the potential of Egb 761 and WG for delaying age-associated tissue degeneration such as sarcopenia in the *C. elegans* model and to test the hypothesis that age-associated sarcopenia can be modulated by certain life-span-extending drugs. Using a combination of fluorescent and transmission electron microscope (TEM) techniques as well as behavioral assays, we demonstrate that both Egb 761 and WG delay age-dependent muscle cell degeneration. The onset of their effects on age-related decline of muscle deterioration and locomotory behaviors is different in aging worms. The insights from using Egb 761 and WG extract on muscle deterioration in the nematodes may provide a basis for developing pharmacological interventions for human sarcopenia.

**METHODS**

**Herbal Extract and Reagents**

Schwabe Pharmaceuticals (Karlsruhe, Germany) provided the standardized *Ginkgo biloba* leaf extract Egb 761. WG extract from *Panax quinquefolius* was a gift from Dr. I. Khan at the University of Minnesota, National Center for Natural Product Research. The stock solutions of both herbal extracts were dissolved in 100% ethanol (EtOH) and further diluted into the worm food to get final concentrations of (100 μg/mL). The final concentration of EtOH did not exceed 0.01% in the food, which is used as a vehicle control.

**Strains of C. elegans**

Wild type *C. elegans* (N2, Bristol) was obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis). Transgenic mutant strain PD4251, which carries an integrated myo-3::gfp transgene in body wall muscle cells, was a gift from Dr. Monica Driscoll at the State University of New Jersey. Assessments of life span, motility, and pharyngeal pumping were performed on solid nematode growth medium (NGM), with a spot of 100 μL of *Escherichia coli* OP50 for food (38). Reproductive adults were transferred to fresh NGM plates and allowed to lay eggs for 2–4 hours, producing age-synchronized groups. All worms were cultivated at 20°C in a temperature-controlled incubator. Egb 761 or WG was added directly to the OP50 food source to feed the worms.

**Fluorescent Microscopy and Quantitation of GFP Reporter Gene Expression**

Age-synchronized PD4251 transgenic nematodes (about 24 in each treatment group) were transferred to a new plate every other day. At different ages, the worms were picked and placed on a glass slide. Live worms were embedded within 2% agarose pads, and a GFP reporter gene expression was examined directly under a fluorescent microscope. Epifluorescent images were acquired at the same exposure parameter using the 40× objective of a microscope (BX 60; Olympus, Tokyo, Japan) equipped with a digital camera (Micropublisher 5.0; QIMAGING, Burnaby BC, Canada). For quantifying a population of GFP reporter animals, each 40× image was analyzed using Image-ProPlus 4.51 software (Media Cybernetics, Silver Spring, MD).

**Electron Microscopy of Muscle Ultrastructure of the Worms**

Aged animals (3 and 11 days) were fixed for 2 hours in 2.0% formaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0), rinsed with distilled water, then postfixed for 45 minutes in cacodylate-buffered (pH 7.0) 1% osmium tetroxide; dehydrated with an ethanol series (50%, 70%, 85%, 95%, and 100%) followed by acetone; infiltrated in ERL 4206 resin (resin contains nonenyl succinic anhydride [26 g], vinyl cyclohexene dioxide [10 g], diglycidyl ether [6 g], and dimethylaminoethanol [0.2 g]); and cured at 70°C for 36 hours. Ultrathin sections (~90 nm) were taken with a Porter-Blum MT-2B microtome (Ivan Sorvall, Inc., Newton, CT) and a diamond knife (Diatome-US, Fort Washington, PA), and were collected on 200-mesh copper grids. Grids were stained with lead citrate and 2% aqueous uranyl acetate. Sections were viewed using a Zeiss EM 109-T TEM (Carl Zeiss, Inc., Thornwood, NY). Results were obtained from representative sections taken from a total of 3 worms in each treatment.

**Life-Span Assay**

Age-synchronized worms fed with different agents were maintained at 20°C. The day of synchronized egg hatching was defined as day 1. The synchronized worms were transferred to fresh plates (30 nematodes per plate) at day 3 after hatching. When the worms started laying eggs, they were transferred daily for 4–5 consecutive days until the cessation of egg laying to avoid overlapping generations. After that, the worms were transferred every other day. Worms were scored as dead if they did not display pharyngeal pumping and respond to touch stimulus.

**A, B, C Motion Scoring**

Locomotive behaviors were scored in three categories as described by Herndon (15). “A” motion is defined as spontaneous movement and significant coordinated sinusoidal locomotion in response to prodding. “B” motion is
decrease of the number of fluorescent nuclei was slower to
open bars). In the worms fed with EGb 761, the rate of
(day 4, 7, 10, 15; Figure A left panels Ctrl, and Figure B
a linear decline of the fluorescence signal as the worms age
scope (15). As represented in Figure 1A and B, there is
can be detected and counted under the fluorescent micro-
progressive decline in the number of GFP-labeled nuclei, which
is localized in body wall muscle nuclei. The age-associated
worm PD4251 expresses a GFP-tagged MYO-3 protein that
examine the muscle degeneration in living animals by using
EGb 761 would improve age-associated tissue deterioration,
(15). To determine if life-span-extending chemicals such as
change in the nuclear distribution of a muscle-specific GFP
muscle structure in living
C. elegans
is an aging-related
muscle cells is alleviated by EGb 761 and
ginseng treatment
One recently developed noninvasive method for assessing
muscle structure in living
C. elegans
is an aging-related
change in the nuclear distribution of a muscle-specific GFP
(15). To determine if life-span-extending chemicals such as
EGb 761 would improve age-associated tissue deterioration,
we first used GFP-tagged
myo-3
gene transgenic worms to
eXamine the muscle degeneration in living animals by using
a fluorescent microscope. The transgenic GFP reporter
worm PD4251 expresses a GFP-tagged MYO-3 protein that
is localized in body wall muscle nuclei. The age-associated
muscle cellular deterioration can be reflected by a pro-
gressive decline in the number of GFP-labeled nuclei, which
can be detected and counted under the fluorescent micro-
scope (15). As represented in Figure 1A and B, there is
a linear decline of the fluorescence signal as the worms age
(day 4, 7, 10, 15; Figure A left panels Ctrl, and Figure B
open bars). In the worms fed with EGb 761, the rate of
decrease of the number of fluorescent nuclei was slower to

Body Bend and Pharyngeal Pumping Assay
The worms at days 5, 8, 11, and 14 were selected for
measurement of the locomotory rate by scoring the number
of body bends in 20 seconds and pharyngeal pumping within
1 minute. Each 25 control or treated adult (3 day old) worms
were transferred to fresh plates and examined at day 5, day 8,
day 11, and day 14. The animals were maintained by trans-
ferring every day (during reproductive period) or every other
day until subjected to the experiment. On the experiment day,
10 selected, age-matched animals were examined for pha-
ryngeal pumping. The same worms were then picked and
washed twice with S basal buffer and transferred to 10 cm
Petri plates seeded with bacteria lawns. The number of body
bends was recorded in 20-second intervals after the animals
had been on the plate for 10 minutes. To keep consistency,
each animal was gently tapped at the back with a platinum
loop. The stimulus was sufficient to induce their response but
did not cause any damage to the tapped region. In all cases,
plates were coded so that the experimenter was blind to
control or treated animals.

Statistical Analysis
Statistical comparison between treatments was done with
an unpaired Student t test using Origin 6.0 software
errors of the mean were used in the figures. Differences with
p < .05 were defined as statistically significant.

RESULTS
Age-Associated Deterioration of Body Wall
Muscle Cells Is Alleviated by EGb 761 and
Ginseng Treatment
One recently developed noninvasive method for assessing
muscle structure in living
C. elegans
is an aging-related
change in the nuclear distribution of a muscle-specific GFP
(15). To determine if life-span-extending chemicals such as
EGb 761 would improve age-associated tissue deterioration,
we first used GFP-tagged
myo-3
gene transgenic worms to
eXamine the muscle degeneration in living animals by using
a fluorescent microscope. The transgenic GFP reporter
worm PD4251 expresses a GFP-tagged MYO-3 protein that
is localized in body wall muscle nuclei. The age-associated
muscle cellular deterioration can be reflected by a pro-
gressive decline in the number of GFP-labeled nuclei, which
can be detected and counted under the fluorescent micro-
scope (15). As represented in Figure 1A and B, there is
a linear decline of the fluorescence signal as the worms age
(day 4, 7, 10, 15; Figure A left panels Ctrl, and Figure B
open bars). In the worms fed with EGb 761, the rate of
decrease of the number of fluorescent nuclei was slower to

some extent compared with untreated ones (Figure 1A).
Quantitative analysis of GFP-labeled nuclei in PD4251
worms revealed (Figure 1B) that both EGb 761 and WG
extracts were able to delay the progression of muscle
degeneration in aged worms. Apparently, the effect of EGb
761 on the worm muscle cells started in midlife and exerted
a significant effect on sarcopenia at day 10, compared with
age-matched control worms (Ctrl 51.4 0.8, vs EGb
55.7 1.9; total worms 24, p = .004) or worms fed with
ginseng (Ctrl 51.4 0.8 vs WG 52.0 2.0 [p = .22]).
However, the effect of WG on muscle cell deterioration appeared relatively late, and the significant effect was obvious at day 15 (Ctrl 37.6 ± 2.3 vs WG 46.0 ± 1.9 [p = .001] vs EGb 39.3 ± 1.5 [p = .4]).

Aside from the fluorescence assay, we used TEM to analyze ultrastructural changes of body wall muscle cells under different treatments, as a visual support of the data presented in Figure 1. Age-related decline in sarcomere integrity was demonstrated dramatically as viewed by TEM (15). The muscle sarcomere integrity of a young worm is shown in Figure 2A. White arrows indicate sarcomeres interfacing with dense bodies (black arrows). Compared with the young worm, a representative TEM section of a 11-day-old worm exhibits disintegrating sarcomeres (white arrow), revealed as disorganized and with reduced dense bodies (Figure 2B). In age-matched worms fed with EGb 761, the muscle integrity viewed by TEM did not exhibit remarkable differences in comparison with the vehicle-fed, 11-day-old controls (Figure 2C). However, the worms (11 day) fed with WG (Figure 2D) displayed an ultrastructural change similar to the young, 3-day-old animals (Figure 2A). Results were obtained from representative sections (at least 8) taken from a total of 3 worms in each treatment.

Figure 2. Representative transmission electron micrographs of the worm’s body wall muscle ultrastructure. A, Muscle structure integrity of untreated worms at day 4. White arrows indicate sarcomeres interfacing with dense bodies (black arrows). B–D, Muscle structure integrity of day 11 worms fed with vehicle (B), EGb 761 at 100 μg/mL (C), or Wisconsin Ginseng (WG) at 100 μg/mL (D) starting at day 1. Results were obtained from representative sections taken from a total of 3 worms in each treatment. Scale bar = 1.0 μm.

Age-Associated Declines of Locomotive Behavior Are Reduced by EGb 761 and WG Extracts

The body movement of young adult worms exhibits a spontaneous, well-coordinated, sinusoidal pattern. The motor activity is reduced with increasing age: the aging animals move slowly and ultimately stop moving. Herndon and colleagues (15) established a behavioral phenotype analysis to characterize locomotion in aging worms. In brief, they sorted worms into three classes. The “A” locomotory class stands for highly mobile worms, the “B” locomotory class represents worms with significantly reduced mobility, and members of the “C” class are immobile individuals. It was found that all animals begin adulthood in the A class, then proceed into the B and finally the C class prior to death (15). The A, B, C locomotion scoring allowed us to look closely at effects of drugs on the development of sarcopenia at the behavioral level. Because the development of locomotory defects in aging worms always follows the order A→B→C, we categorized worms via counting the number of worms falling into corresponding classes and reclassified them every other day until all animals had died.

Both EGb 761 and WG extracts (100 μg/mL each, fed from day 1 of age until the worms were collected) altered
the ratio of the number of worms in a given category (A, B, or C) to the total number of worms present at the start of the experiment from midlife to the late stage of life (Figure 3A). Among those behavioral phenotypes, the behavior in A motion is particularly interesting, for it represents highly mobile worms that still retain good muscle function or only suffer mild locomotory deficits without evidence of significant muscle deterioration. We reasoned that if the treatment with EGb 761 could attenuate sarcopenia resulting in delaying the onset of age-related muscle deterioration, the fraction of A class animals would remain higher in drug-treated nematodes in than in untreated animals. As expected, the statistical analysis showed that there was a significant increase in the fraction of A class animals treated with EGb 761 or WG on the given days (Figure 3B). Interestingly, EGb 761 significantly delayed the onset of locomotory impairment and retained more A motion animals in midlife (day 10, p = .016; day 12, p = .0007; day 14, p = .003, total control worms: n = 118, total worms fed with EGb 761: n = 114). The significant effect of WG on the development of sarcopenia is at days 12, 14, and 16 (day 12, p = .006; day 14, p = .004; day 16, p = .047; total control worms: n = 118; total worms fed with WG: n = 118). The pattern of EGb 761 and WG affecting sarcopenia in the worms over age

Figure 3. Age-related locomotor behavior decline and effects of treatment. A, Worms at indicated ages were untreated (control; C), fed EGb 761 (at 100 µg/mL; E) or fed Wisconsin Ginseng (WG; at 100 µg/mL). A, Four different classes of locomotory behaviors (A, B, C, as described in "Methods"; dead animals classified as D) in the worms at different ages. Fraction is calculated as the remaining number of animals in a given category (A, B, C, or D) counted on the day indicated over the total number of animals present at the start of the experiment. Fraction of dead worms on any given day is inclusive of counts of all dead worms from previous days. B, Statistical analysis of scores of A motion only. Fraction is calculated as the number of animals in A motion counted on the given days over the total number of animals present at the start of the experiment. Locomotory decline of A motion was significantly delayed by both EGb 761 and WG in the worms at 12, 14, and 16 days, compared with the age-matched worms without treatment (*p < .005, **p < .001; 118 worms in each treatment condition). C and D, Dose response of A motion in worms fed increasing concentrations of EGb 761 (C) or WG (D).
correlates with that observed in the structural assays by quantitative GFP fluorescence (Figure 1B). Furthermore, no dose response of EGb 761 and WG in A motion was observed among concentrations tested (10–100 μg/mL) suggesting that concentrations < 1 μg/mL might have been effective (Figure 3, C and D).

Declines of Locomotory Rate and Pharyngeal Pumping in Aged Animals Are Reduced by Treatment of EGb 761 or WG

The age-associated declines in locomotory behavior and pharyngeal pumping are positively correlated with each other and with aging (39). We note that the onset of sarcopenia appears at different times among individual animals and that the progressive decline of muscle function proceeds at variable rates in aging nematodes. For this reason, a defined category of animals (A motion) was chosen to assess the locomotory rate and pharyngeal pumping (10 worms were selected of 25 animals, if death occurred from the corresponding plate) on the given day. The locomotory rate was measured by examining the number of body bends in 20 seconds at different time points. Figure 4A shows that only ginseng significantly reduced the age-related decline of the number of body bends at day 14 (n = 50, p = .007). Surprisingly, there is no significant improvement of locomotory rate by EGb 761 treatment on any given day (n = 50, p = .175).

Progressive muscle deterioration not only occurs in body wall muscles, but also in pharyngeal muscles in C. elegans (15,19). The pharynx is a neuromuscular organ that displays rhythmic contractions. The pumping rate was measured by counting the number of contractions in 20 seconds. Figure 4B shows that both EGb and WG could increase the pharyngeal pumping in the midlife of worms. The significant increase of pumping by EGb 761 treatment was observed at day 11 (n = 40, p = .028), but WG had a significant effect on pumping on a later day (day 14, n = 40, p = .028).

The survival assay, or life-span assay, was conducted next in the same population of worms under defined drug treatments (Figure 4C). Consistent with our previous report, EGb 761 significantly extended the mean life span in the nematode. Treatment of wild-type worms (N2) with EGb 761 extended their mean life span by 9% (p = .018). However, we failed to observe a significant increase of the worm’s mean

---

mean number of body bends at day 14 was significantly reduced in the worms fed WG, but not EGb 761, compared with untreated control worms (p < .05, total number of animals tested with different treatments over time: n = 30 for day 5 or 8, n = 40 for day 11, n = 40 for day 14. Ten animals were assayed in each trial). B. Effect of drug treatment on pharyngeal pumping rates of animals investigated during the body bend experiment. The pumping rate of EGb 761–treated animals at day 10 was significantly delayed at the later day (day 14, n = 40, p = .02). C. Life span of the worms (PD4251) under different treatments. The survival curves compare wild-type worms (N2) with EGb 761 extended their mean life span by 9% (p = .018). However, we failed to observe a significant increase of the worm’s mean

---

Figure 4. Age-associated decline of body bend and pharyngeal pumping. A. Effect of drug treatment on the locomotor rate, assessed by examining body bends, in wild-type (N2) animals at different ages. Graph represents the mean body bend per minute in the untreated worms (white bars), worms fed EGb 761 (black bars), or fed Wisconsin Ginseng (WG; hatched bars). The decline in the
life span by WG ($p = .086$). At the point of 50% survival rate, both EGb 761 and WG show similarity in extending the life span from 15 days to 17 days (Figure 4C).

**DISCUSSION**

The present study describes the efficacy of natural extract EGb 761 and WG to modulate the process of muscle aging, or sarcopenia, and the associated decline in locomotive behaviors. Interestingly, the reduced decline of muscle cell integrity (Figures 1 and 2) by the two extracts displayed onsets at different ages, which seems to correlate with their effects on age-dependent decline in motor behavior (Figures 3 and 4). These data support our hypothesis that age-associated sarcopenia can be modulated by certain life-span-extending drugs.

Both EGb 761 and WG have long been known to have anti-aging properties (40). Despite their different chemical components and applications, they share some common features including anti-oxidative, anti-inflammatory, and anti-apoptotic properties (41,42). Their common features may explain a general anti-aging mechanism. It is well known that oxidative stress plays an important role in aging (43). Studies have suggested that oxidatively damaged macromolecules are increased overall in skeletal muscle tissue during ageing (44), which has been correlated with a reduction in anti-oxidative capacity (45). Skeletal muscle is a postmitotic tissue that lacks high repair capacity. Lower levels of anti-oxidative defense in aging satellite cells, which are usually recruited to replace damaged fibers, may negatively affect the potential regeneration of muscle fibers (46,47). Aiken and colleagues (48) reported that mitochondrial DNA deletions subsequent to oxidative damage result in dysfunction of the electron transport system and an abnormal energy supply in the affected segment of skeletal muscle, which will cause muscle fiber breakage and loss as observed with aging. Taken together, the oxidative insult accumulated in the skeletal muscle with the aging process may be an important factor in sarcopenia. A positive correlation of anti-oxidative stress and longevity in *C. elegans* has been well established (49). The interventions aimed at preventing oxidative damage and environmental stresses has been shown to extend life span in worms (24,25,50–52). Nevertheless, chemicals other than anti-oxidants also extend life span in *C. elegans* (23,53), suggesting that multiple mechanisms exist.

Although EGb 761, but not WG, extends mean life span by the survival assay, the actual survival curves overlap at the mean life span (Figure 4). Thus both EGb 761 and WG modestly increased mean life span. It should be noted that EGb 761 has an effect at midlife onward, and WG later in life (Figure 1), and their life-span curves fit this idea. What might differ between WG and EGb 761 that accounts for the different onset of their effects? Ginsenoside has been known to be a typical “adaptive” chemical, i.e., it is able to work bidirectionally, depending on the immediate environment. For example, in patients with high blood pressure, it reduces it, whereas the same chemical increases it in patients with low blood pressure. This property of ginsenosides may explain why WG had a late onset in its effect on aging and sarcopenia.

The specific protective mechanisms of EGb 761 and WG against age-related cell deterioration are unknown, partially due to the complexity of their makeup. However, studies of active constituents of the extracts implicate some molecular mechanisms of action. Among active ingredients of Panax ginseng, ginsenosides Rg3 and Rh2 are major inhibitors of Ca$^{2+}$ channels and show some Ca$^{2+}$ channel selectivity (54). Rg3 is also a novel Na$^{+}$ channel inhibitor capable of acting on the resting and open states of Na$^{+}$ channels via interactions with the S4 voltage-sensor segment of domain II (55). Furthermore, some ginsenosides as well as ginsenoside metabolites can influence 5-HT3A receptor channel activity in *Xenopus* oocytes (56) and modulate excitatory (N-methyl-D-aspartic acid [NMDA]) and inhibitory (gamma-aminobutyric acid [GABA]) neurotransmitter receptors (57). In contrast, the protective mechanism of EGb 761 can be attributed to a range of biochemical and pharmacological effects (28), including ginkgolide B as an antagonist of platelet-activating factor (PAF) receptor (33), enhancement of neuronal plasticity (58), anti-inflammatory effects (59), and anti-apoptotic effects in neuronal cells (60,61). Accumulating evidence suggests that many of the actions of EGb 761 are so-called “polyvalent” actions, that is, the therapeutically effective EGb 761 is the net effect of interactions among various biological activities of the individual substances in the extract. Presumably, this is one of the advantages of using chemicals obtained from natural products for the prevention and treatment of infirmity, as well as the maintenance of health (62). Several lines of experimental evidence indicate that the development of sarcopenia is not attributed to one or two contributors in its pathogenesis, rather, it is most likely the result of interacting actions of multiple intrinsic and extrinsic factors (63,64). It has been proposed that a number of factors might play important roles in the onset and progression of sarcopenia with advancing age. These factors include deficient satellite cell recruitment (65,66), a variety of hormonal changes (67,68), oxidative stress, mitochondrial DNA (mtDNA) damage (69–71), apoptosis (69,72), inadequate nutrition (10,73), and reduced physical activity (74,75). Nevertheless, oxidative stress is one of the most important factors. The evidence is that the stressed *C. elegans* models also exhibit accelerated sarcopenia. Experimental evidence indicates that age-related behavior declines were delayed in *C. elegans* mutants in the daf-2/insulin-like pathway, which governs longevity and has reduced oxidative stress (19). Similar results were observed in other stress models such as in *Drosophila*, mammals, and humans (76–78).

The relationship between specific tissue maintenance and life span has a significant impact on the treatment on life span versus muscle integrity over time. Because sarcopenia could predict life span, that is, a positive correlation between sarcopenia and life span, pharmacology of life span would have to start early enough to overcome age-associated muscle integrity decline. However, it is not realistic to use drugs beginning at an early age for the long term to enhance life span. Lifestyle change is certainly a better approach, except for genetic disorders of aging, or premature aging, for which long-term use of pharmacological therapy would be necessary.
ACKNOWLEDGMENTS
This study was supported by IPSEN (Paris, France) and partially by National Institutes of Health (NIH) grant R01AT001928-04 (NIH National Center for Complimentary and Alternative Medicine; YL).

We thank Dr. Catherine Wolkow from the National Institute on Aging for insightful discussion and Marishka Brown for reading and commenting on the manuscript.

CORRESPONDENCE
Address correspondence to Yuan Luo, PhD, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 20 Pine St., PH501, Baltimore, MD 21201. E-mail: yluo@rx.umaryland.edu

REFERENCES