석사학위 논문

포스파티딜세린의 스트레스 반응, 노화에 미치는 영향 및 기전연구

Effect of phosphatidylserine on stress response and aging and its underlying mechanisms in *Caenorhabditis elegans*

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의료과학과

김보경

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지도교수 박 상 규

이 논문을 이학석사학위 논문으로 제출함

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순천향대학교 대학원
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2020년 12월

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Contents

List of Figures

Lists of Tables

ABSTRACT

Effect of phosphatidylserine on stress response and aging and its underlying mechanisms in *Caenorhabditis elegans*

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Phosphatidylserine is one of phospholipids comprising plasma membranse and rich in brain and nervous system. Phosphatidylserine content is reduced with aging and age-related decrease in phosphatidylserine is known to contribute to cognitive impairment and Alzheimer's disease in the elderly. In this study, we investigated the effect of dietary supplementation with phosphatidylserine on stress response and aging in *C .elegans in vivo*. Treatment with phosphatidylserine significantly increased resistance to oxidative stress. It also significantly extended-lifespan accompanying reduced fertility as a trade off. Age-related decline in motility was specially delayed by phosphatidylserine. Dietary supplementation with phosphatidylserine increased cellular levels of reactive oxygen species (ROS) and induced expression of stress-responsive

genes, *hsp-16.2* and *sod-3*. These results suggested that the effect of phosphatidylserine may be through a hormetic effect. Extended lifespan by phosphatidylserine specifically overlapped with that by reduced insulin/IGF-1-like signaling and required DAF-16. Phosphatidylserine ameliorated amyloid-beta induced toxicity in Alzheimer's disease model. Reduced survival by high-glucose diet was retared by dietary supplementation with phosphatidylserine. We report anti-oxidative stress and anti-aging activities of the phosphatidylserine for the first time *in vivo* and propose a possible underlying mechanism.

Ⅰ. Introduction

Aging is characterized as accumulation of diverse deleterious changes in cells and tissues, and increased risk of diseases and death as a result (Pignolo 2018). Several theories have been proposed to explain the mechanisms of aging. Among various theories, the free radical theory is one of most widely-studied one. The free radical theory suggests that numerous free radicals cause cellular damage and accumulation of damage finally leads to aging. Free radicals can be produced by environment pollution, cigarette, and ultraviolet. Major free radicals are reactive oxygen species (ROS), the byproduct of mitochondrial electron transport chain reaction (Harman 1956). Mitochondiral theory of aging focuses on role of mitochondrial dysfunction. This theory is closely linked to the free radical theory of aging, but also involved with genetic and bio-energetic alterations within cells (Harman, 1972). Mitochondria generate cellular energy, produce reactive oxygen species (ROS) as a by product (Angelova & Abramov, 2016), and involve programmed cell death (Galluzzi, Kepp, Trojel Hansen, & Kroemer, 2012). According to this theory, aging of cells is due to constant delivery of ROS inside mitochondria, which causes damage to mitochondrial DNA. Mitochondirial DNA is more vulnerable than nuclear DNA due to lack of histones and repairing enzymes (Didier Morin, 2018). The damaged mitochondrial DNA leads to deficiency of key enzymes involved in electron transport chain reaction and increased ROS generation as a result. (Fariss, Chan, Patel, Van Houten, & Orrenius, 2005). The telomere theory suggests that the attrition of telomere sequences at chromosome end plays a key role in aging (Kunlin, 2010). However, there is no single theory of aging that can explain all phenomena observed with aging. Many of the proposed theories interact with each other in a complex way (Kunlin, 2010).

Based on the free radical theory of aging, many genetic or dietary

interventions that modulate cellular anti-oxidant defense system have been studied. Transgenic animals carrying an additional copy of catalase (CAT) or superoxide dismutase (SOD) did not show lifespan extension (Seto et al. 1990; Orr and Sohal 1994). However, simultaneous over-expression SOD and CAT delayed aging process and extended lifespan in *Drosophila melanogaster* (Bayne and Sohal 2002). The extended lifespan by double trans-genes was only seen in short-lived strain, but not in long-lived strains (Orr and Sohal 2003). Transgenic animals carrying an additional copy of mitochondrial CAT showed increased lifespan (Schriner et al. 2005). In fact, effects of genetic manipulations with anti-oxidant genes on lifespan are still elusive.

Effect of dietary intervention have been reported using compounds exhibiting anti-oxidant activity, including resveratrol, curcumin, vitamin E, and cystein derivatives. Dietary supplementation of resveratrol increased the lifespan of yeasts, fruit flies and *C. elegnas* (Howitz et al. 2003; Gruber et al. 2007; Long et al. 2009). A synthetic curcumin derivative showed anti-aging and anti-oxidant activities and reduced amyloid beta (Aβ)-induced toxicity (Lim et al. 2001). Supplementation with vitamin E prevented cognitive impairment and increased lifespan (Mecocci et al. 2000). Cysteine derivatives showed anti-oxidant activity and extended lifespan in *C. elegans* (Oh et al. 2015; Kim et al. 2017; Kim and Park 2017).

Phosphatidylserine is an anionic phospholipid found in biological membrane and plays a important role in brain neuronal function and cellular signaling (Leventis and Grinstein 2010). Phosphatidylserine is known to improve neuronal function including recovery of axon and memory impairment (Lee et al. 2015; Abay et al. 2017). During aging, the level of phosphatidylserine decreases. This is associated cognitive impairment and pathogenesis of Alzheimer's disease (AD) (Cunnane et al. 2012; Suleimanova et al. 2017). Dietary supplementation with phosphatidylserine improved memory and learning, and increased axonal transport in neurodegenerative diseases (Naftelberg et al. 2017).

In this study, we examined effects of phsphatidylserine on aging using *C.*

elegans as a model system. *C. elegans* has a short life-cycle and produces many descendants. Since the entire genome sequence is revealed, it is easy to perform genetic manipulations, such as knockdown, knockout, and over-expression. Due to these advantages, *C. elegans* is a widely used model organism in aging research. We investigated anti-oxidant and anti-aging effect of phosphatidylserine *in vivo*. In addition, effects of phosphatidylserine on age-related physiological changes and age-related diseases were tested. Finally, we identified underlying mechanism involved in lifespan extension using long-lived genetic mutants. This study reveals novel bio-activities of phosphatidylserine and provides a scientific rationale for the development of phosphatidylserine as an anti-aging therapeutic compound *in vivo*.

Ⅱ. Materials and Method

Ⅱ-1. Worm strains and maintenance

All strains were purchased from the *C. elegans* Genetics Center (CGC, Minneapolis/St. Paul, MN, USA). The wild type strain is N2. The green fluorescent protein (GFP) expressing strains, CL2070 (dvIs70 [*Phsp-16.2*::GFP, *rol-6*]) and CF1553 (muIs84 [*Psod3*::GFP, *rol-6*]), were used to study change in expression of *hsp-16.2* and *sod-3*, respectively. The long-lived mutants, *age-1* (*hx546*), *clk-1* (*e2519*), and *eat-2* (*ad465*), were used to reveal underlying mechanism of longevity. CL4176 (dvls27 [*myo-3/Ab1-42/let* UTR, *rol-6*]), expressing transgenic human $A\beta_{1-42}$ gene in muscle tissues was used to investigate the effect of phosphataidylserine on AD. All strains except CL4176 were maintained at 20℃ on solid Nematode Growth Media (NGM) plates (25 mM NaCl, 1.7% agar, 2.5 mg/mL peptone, 50 mM KH_2PO_4 (pH 6.0), 5 ㎍/mL cholesterol, 1 mM $CaCl_2$, and 1 mM $MgSO_4$) spotted with *Escherichia coli* OP50 as a food source. CL4176 strain maintained at 15℃ on solid NGM plate. Phosphatidylserine (SigmaAldrich, St. Louis, USA) was dissolved in 99% ethanol and 100 μL of different concentrations of phosphatidylserine were spread on solid NGM plate. After spreading, OP50 were added and let dried.

Ⅱ-2. Resistance to oxidative stress

To obtain age-synchronized worms, 5-6 young adult worms from the same mother were transferred on solid NGM plate added with OP50 and allowed to lay eggs for 5-6 h at 20℃. Then, all adult worms were removed from the plate and the remaining eggs were hatched for another 3 days at 20℃. Thirty age-synchronized young adults were transferred to fresh NGM plates pre-treated with different concentrations of phosphatidylserine and adapted for 24 h. After 24 h, thirty worms moved to 96-well plate containing 1 mM

hydrogen peroxide (H_2O_2) in S-basal without cholesterol (5.85 g sodium chloride, 1 g potassium phosphate dibasic, and 6 g potassium phosphate monobasic in 1 L sterilized distilled-water) (5 worms/well). The survival of worms was measured after 8 h of incubation. A worm unresponsive to stimulation with a worm picker was counted as dead.

Ⅱ-3. Lifespan assay

To prevent bagging, 5-fluoro-2'-deoxyruridine (12.5 mg/L) was added to NGM plates. Sixty age-synchronized worms were used. The number of alive and dead was recorded until all the worms were dead. Worms lost, killed, or bagged during the assay were excluded from statistical analysis. We employed the log-rank test for statistical analysis (Peto and Peto 1972).

Ⅱ-4. Measurement of fertility

Ten age-synchronized 2-day-old worms were transferred individually to fresh NGM plates with or without phosphatidylserine (100 ㎍/L) and permitted to lay eggs for 24 h at 20℃. Then, ten adult worms were transferred to fresh NGM plates again. The remaining eggs were hatched and grown for 48 h at 20℃. The progeny generated in the remaining plate was counted. Adult worms were transferred to fresh NGM plates everyday until each worm stopped producing progeny.

Ⅱ-5. Locomotion assay

One hundred age-synchronized young adult worms were transferred to NGM plates pre-treated with or without phosphatidylserine (100 ㎍/L) at 20℃. Motility of single worms was monitored on 5, 10, 15, 20, and 25 days after hatching. Motility of each worm was classified into three phases: Phase 1, a

worm moving without any mechanical stimulation; phase 2, a worm that moved in response to mechanical stimuli; phase 3, a worm that moved only its head part with mechanical stimulus. Motility was compared between the untreated control and phosphatidylserine-treated group.

Ⅱ-6. Thrashing assay

Fifteen age-synchronized worms were transferred to fresh NGM plates containing phosphatidylserine (100 ㎍/L) at 20℃. On 5, 10, 15, 20, and 25 days, individual worm was transferred to NGM plates without OP50. After incubating for 2 min to remove *Eschericia coli,* worms were transferred to 1 mL of M9 buffer. After 10 min, the thrashing rate was counted under microscope.

Ⅱ-7. Measurement of ROS

Twenty age-synchronized young adults worms were grown on fresh NGM plates with phosphatidylserine (100 ㎍/L) at 20℃. After 5 and 7 days, worms were incubated in a 96-well black plate containing 190 μL of PBST and 10 μL of H_2DCF-DA (Sigma-Aldrich, St. Louis, USA) for 3 h (1 worm/well). The fluorescence intensity of each well was measured using a fluorescence multi-reader (Infinite F200, Tecan, Grodig, Austria)

Ⅱ-8. Expression of stress-responsive genes

Age-synchronized CL2070 and CF1553 worms grown on NGM plates containing phosphatidylserine (100 ㎍/L). After 7 days, worms were mounted on a slide glass coated with 2% agarose and anesthetized with 1 M sodium aide. Expression of GFP was monitored with a confocal microscope (Olympus FV10i, Olympus, Tokyo, Japan). To quantify GFP expression, a fluorescence

multi-reader was used (Infinite F200, Tecan, Grodig, Austria).

Ⅱ-9. RNA interference (RNAi)

Escherichia coli clones for RNAi were obtained from Ahringer RNAi library (Kamath et al. 2003). To induce the expression of double-stranded RNA, 0.4 mM isopropyl-b-D-thio-galactoside (IPTG, Sigma-Aldrich, St. Louis, MO, USA) was added to culture media. The cultured bacteria were used as a food source for RNAi experiment. Empty vector clone was used as a negative control.

Ⅱ-10. Aβ-induced toxicity

Thirty age-synchronized CL4176 worms were transferred to NGM plates containing phosphatidylserine (100 ㎍/L) at 15℃ and permitted to lay eggs for 2 h. Next, all adult worms were removed from NGM plates and the remaining eggs were hatched and grown for 24 h. Randomly selected sixty worms were incubated at 25℃ for 24 h to induce the expression of human Aβ gene. The number of paralyzed worms were counted every hour until all the worms were paralyzed.

Ⅱ-11. Survival under high-glucose diet (HGD)

Fresh NGM plates containing with 100 μL of 40 mM glucose and 100 ㎍/L of phosphatidylserine were prepared. Survival of worms was recorded every day until all worms were dead.

Ⅲ. Results

Ⅲ-1. Phosphatidylserine increased resistance to oxidative stress.

To determined the anti-oxidant activity of phosphatidylserine, using H_2O_2 that as an oxidative stress inducer *in vivo*. Survival of worms was measured after treatment with different concentrations of phosphatidylserine (Fig. 1). After 8 h under oxidative stress conditions, the mean percent survival of the untreated control group was 47.8 ± 4.01% (mean of three independent experiments ± SEM) and that of phosphatidylserine-treated group (100 ㎍/L) was 78.9 ± 8.68% (P = 0.031). This result showed that dietary supplementation with phosphatidylserine can increase resistance to oxidative stress. However, lower and higher concentration of phosphatidylserine did not increase resistance to oxidative stress (Fig. 1). Taken together, we determined that the optimum concentration for anti-oxidant activity of phosphatidylserine was 100 ㎍/L in *C. elegans*.

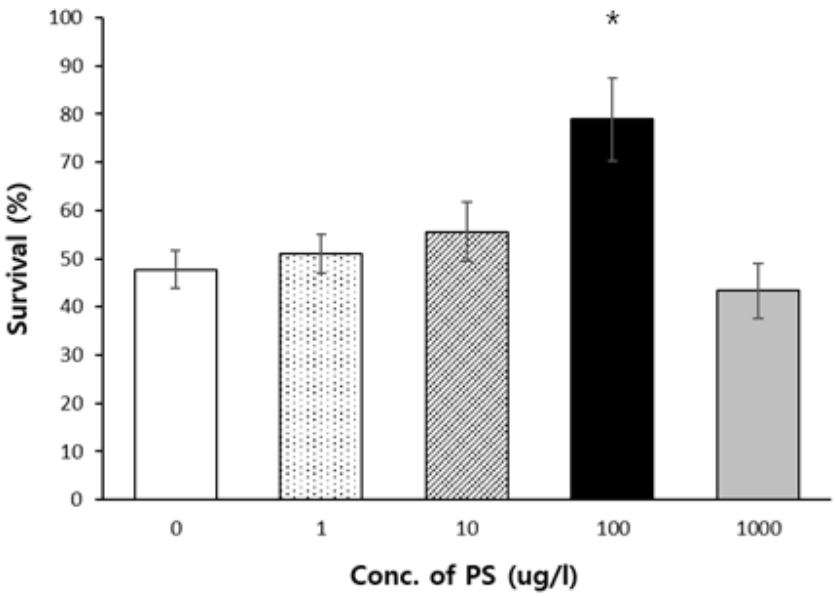

Figure. 1 Effect of phosphatidylserine on response to oxidative stress. Survival under oxidative stress induced by H_2O_2 was determined with different concentrations of phosphatidylserine. PS, phosphatidylserine; *, statistically significant ($P < 0.05$).

Ⅲ-2. Phosphatidylserine extended lifespan.

The free radical theory of aging suggests that accumulation of cellular damages caused by oxidative-stress is one of major causal factors of aging (Harman, 1956). Based on this theory, we investigated whether the anti-oxidant activity of phosphatidylserine lead to lifespan extension. As shown in Fig. 2, phosphatidylserine (100 μg/L) significantly increased both mean and maximum lifespan. Mean lifespan of untreated group was 18.5 days and that of phosphatidylserine- treated group was 19.8 days (P = 0.002). In addition, maximum lifespan increased from 22 days in untreated control to 25 days in phosphatidylserine-treated group. Independent repetitive experiments showed similar significant increases in lifespan (Table 1). These results suggest that phosphatidylserine has a lifespan-extending activity possibly through its anti-oxidant activity in *C. elgans*.

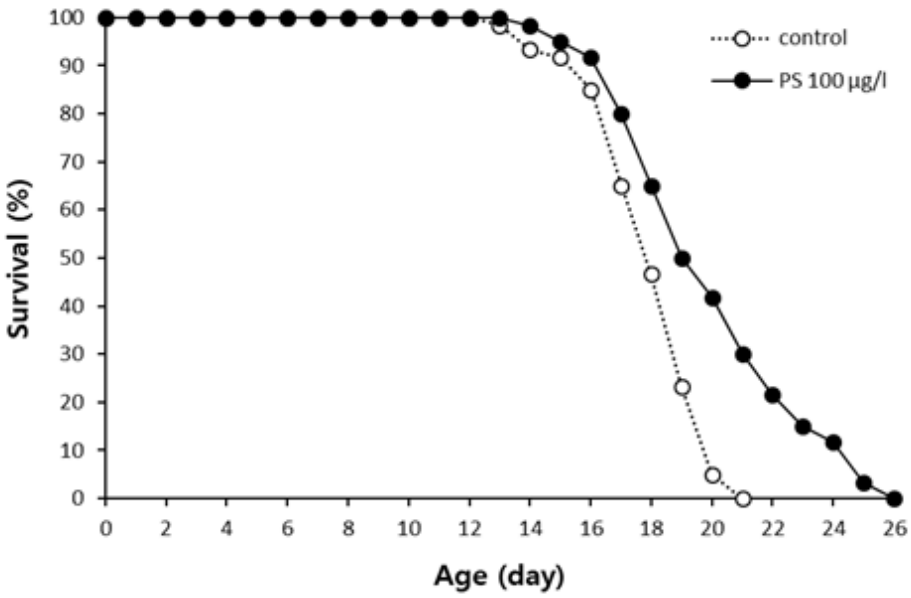

Figure 2. Effect of phosphatidylserine on lifespan. Lifespan of untreated control was compared with phosphatidylserine-treated group. PS, phospahtidylserine.

Table 1. Effect of phosphatidylserine on lifespan.

	PS (μg/l)	No. of animals used	Lost or censored	Scored	Mean lifespan (day)	Maximum lifespan (day)	*P* value[a]
1st experiment	0	60	2	58	18.5	22	
	100	60	1	59	19.8	25	0.002
2nd experiment	0	60	1	59	16.6	20	
	100	60	0	60	18.2	25	0.001
3rd experiment	0	60	0	60	17.1	20	
	100	60	0	60	19.0	25	< 0.001

[a]*P* value was calculated using the log-rank test by comparing the survival of the untreated control group (0 μg/L phosphatidylserine) to that of the phosphatidylserine-treated group (100 μg/L phosphatidylserine).

PS, phosphatidylserine.

Ⅲ-3. Fertility was reduced by supplementation with phosphatidylserine.

Previous studies have proposed that many lifespan extending interventions reduced fertility as a trade-off (Johnson 1990; Gruber et al. 2007). Therefore, we investrigated the effect of phosphatidylserine on reproduction of *C. elegans*. In untreated control, total number of progeny produced during the whole gravid period was 244.3 ± 5.61 (mean of 10 worms ± SEM). Phosphatidylserine-supplemented group produced 204.6 ± 9.84 progeny (P = 0.001) (Fig. 3). The time-course distribution of progeny during gravid period showed significant decreases in the number of progeny on 2 and 3 days after hatching (Fig. 4). On 2 days after hatching, the numbers of progeny were 26.8 ± 1.90 and 22.1 ± 1.67 in the untreated control and phosphatidylserine-treated group, respectively (P = 0.044). The progeny produced by 3-day-old worms decreased from 131.8 ± 4.85 in the untreated control to 100.7 ± 9.14 in the phosphatidylserine-treated group (P = 0.007). Independent replicative experiments also showed reduction in fertility by supplementation with phosphatidylserine. These findings indicate that dietary supplementation with phosphatidylserine extends lifespan accompanying reduced fertility as a trade-off.

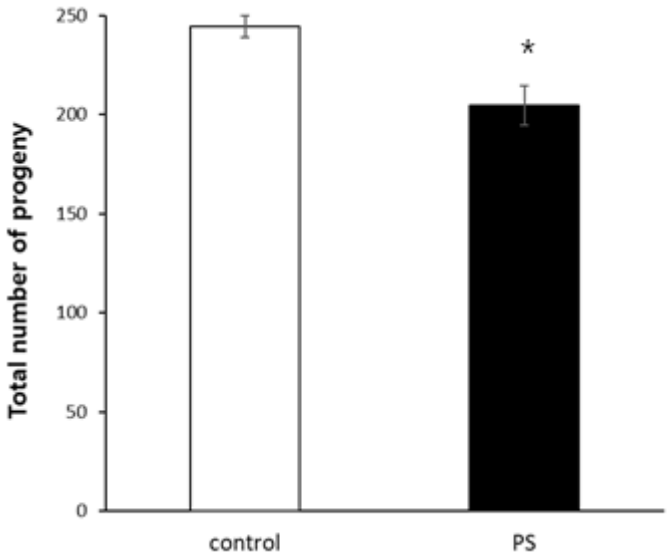

Figure 3. Reduced fertility upon supplementation with phospahtidylserine. Total number of progeny produced during gravid period was compared between the untreated and phosphatidylserine-treated group. PS, 100 μg/L of phosphatidylserine; *, statistically significant ($P < 0.05$).

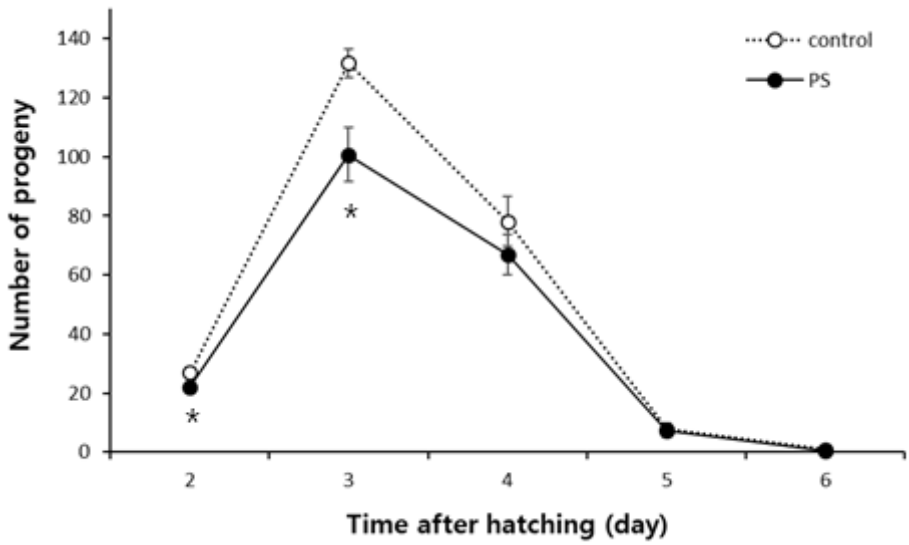

Figure 4. Time-course distribution of progeny during gravid period. Time-course distribution of progeny during gravid period .is shown. PS, 100 μg/L of phosphatidylserine; *, statistically significant ($P < 0.05$).

Ⅲ-4. Age-related decline in motility was retarded by phosphatidylserine

Age-related changes in muscle include muscle atrophy and reduced motility. Locomotive behavior declines with aging in *C. elegans*. We examined the role of dietary supplementation with phosphatidylserine on age-related decline of motility. In 5- and 10-day-old worms, no clear difference in locomotion was observed between untreated control and phosphatidylserine-treated group (Fig. 5). In 15-day-old control group, the percentage moving spontaneously without any mechanical stimuli (phase 1) decreased to 58.5 ± 1.06% (mean of three independent experiments ± SEM). In contrast, in 15-day-old phosphatidylserine-treated group, the percentage of phase 1 was still 79.1 ± 3.36 ($P = 0.004$). A statistically specially difference between the untreated control and phsophatidylseirne-treated groups was also observed in 20-day-old worms. More worms were categorized as 'phase 3', which includes worms moving only their head by mechanical stimulation in the untreated control: 74.2 ± 3.53% in the untreated control and 53.9 ± 5.23% in the phosphatidylserine-treated group ($P = 0.033$) (Fig. 5).

For quantitative analysis of motility, we performed thrashing assay (Fig 6). In 5-day-old worms, dietary supplementation with phosphatidylserine significantly increased the thrashing rate to 124.3 ± 2.37, compared to untreated control (113.8 ± 1.03, $P < 0.001$). The thrashing rate in 10-day-old worms was also increased by phosphatidylserine supplementation: 97.9 ± 4.17 in the untreated control and 126.2 ± 3.48 in the phosphatidylserine-treated group ($P < 0.001$). These qualitative and quantitative analyses age-related decline in motility suggest that phosphatidylserine delayed age-related decline of motility in *C. elegans*.

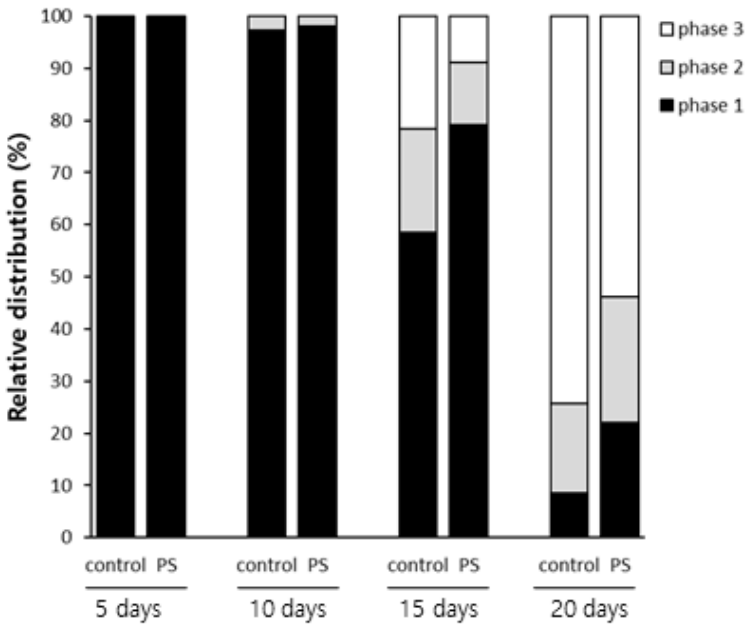

Figure 5. Effect of phosphatidylserine on age-related decline in motility. Worms wre classified according to their locomotive activity: Phase 1, a worm moved spontaneously without any stimulation; Phase 2, a worm moved only under mechanical stimulation; Phase 3, a worm moved only tis heatd in response to mechanical stimulation. PS, 100 μg/L of phosphatidylserine.

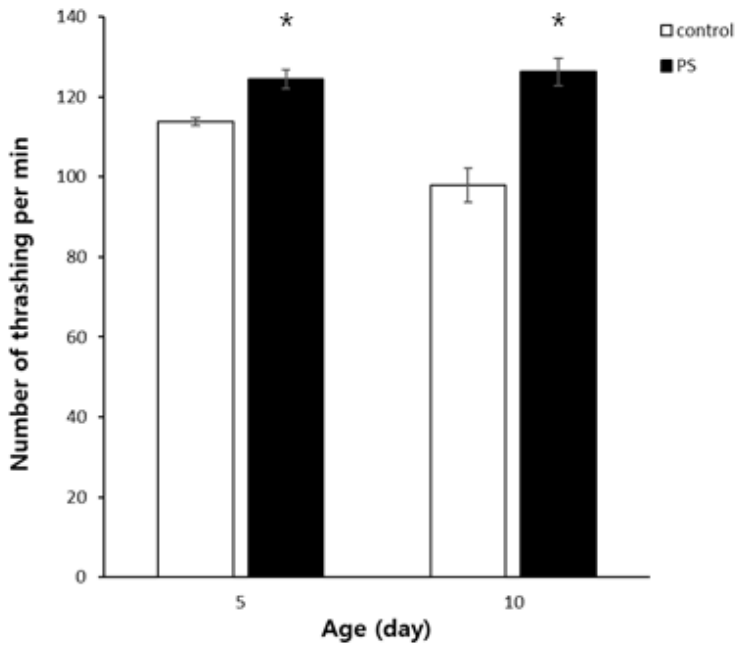

Figure 6. Effect of phosphatidylserine on thrashing. Quantification of motility was measured by counting number of thrashing per min in the untreated and phosphatidylserine-treated worms. PS, 100 μg/L of phosphatidylserine; *, statistically significant ($P < 0.05$).

Ⅲ-5. Phosphatidylserine increased cellular ROS level

The free radical theroy of aging suggests that the major cause of aging is accumulation of oxidative damage induced by cellular ROS (Harman, 1956). In the previous experiments, we found that resistance to oxidative stress and lifespan can be extended following supplementation with phosphatidylserine. Then, we hypothesized that these anti-oxidant and lifespan-extending activities of phsopatidylserine could be resulted from its ROS scavenging activity. Surprisingly, our data showed that cellular ROS levels were rather increased by supplementation with phosphatidylserine (Fig 7). Phospatidylserine-treated groups showed increased cellular ROS levels compared with untreated control: 78 ± 4.1% increase on 5 days and 58 ± 4.9% increase on 7 days ($P < 0.001$) and 44 ± 4.6% increase on 9 days after hatching ($P < 0.001$).

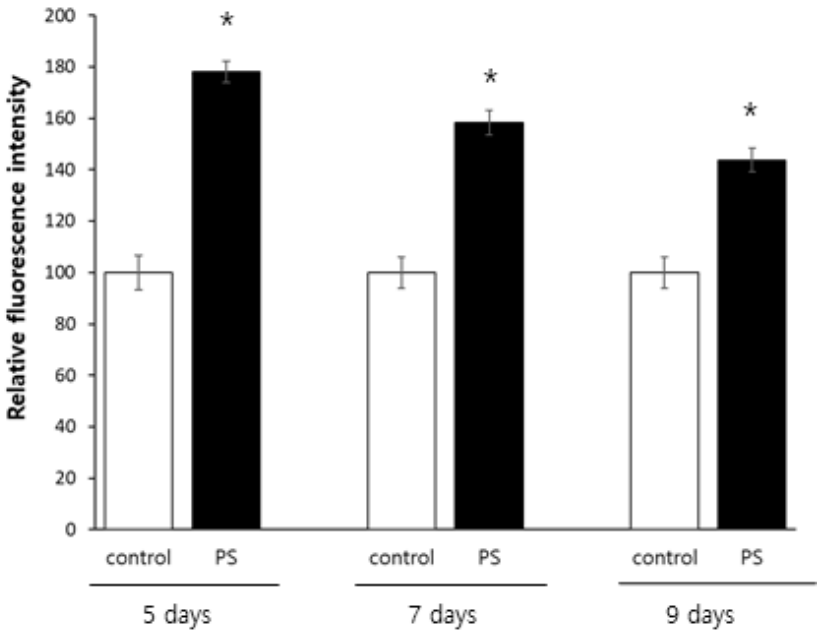

Figure 7. Increased cellular levels of ROS following supplementation with phosphatidylserine. Relative fluorescence intensity in the phosphatidylserine-treated group was compared with the untreated control on the indicated days after hatching. PS, 100 ㎍/L of phosphatidylserine; *, statistically significant ($P < 0.05$).

Ⅲ-6. Stress-responsive genes were induced by phosphatidylserine

After observing increased ROS by phosphatidylserine, we asked whether dietary supplementation with phosphatidylserine worms induce stress-responsive genes. *hsp-16.2*, a heat shock responseive gene, and *sod-3*, an anti-oxidant genes, were used for this experiment. Expression of both *hsp-16.2* and *sod-3* were induced by phosphatidylserine treatment (Fig. 8). The relative fluorescence intensities induced by *hsp-16.2* promoter were 100.0 ± 5.42 in untreated control group and 137.0 ± 3.84 phosphatidylserine-treated group ($P < 0.001$) (Fig. 9). The expression of *sod-3* was also increased up to 124.0 ± 17.69% ($P < 0.001$) by phosphatidylserine treatment. These finding suggest that dietary supplementation with phosphatidylserine accelerate increase in cellular ROS levels and enhances expression of stress-responsive genes to adapt to pro-oxidant conditions.

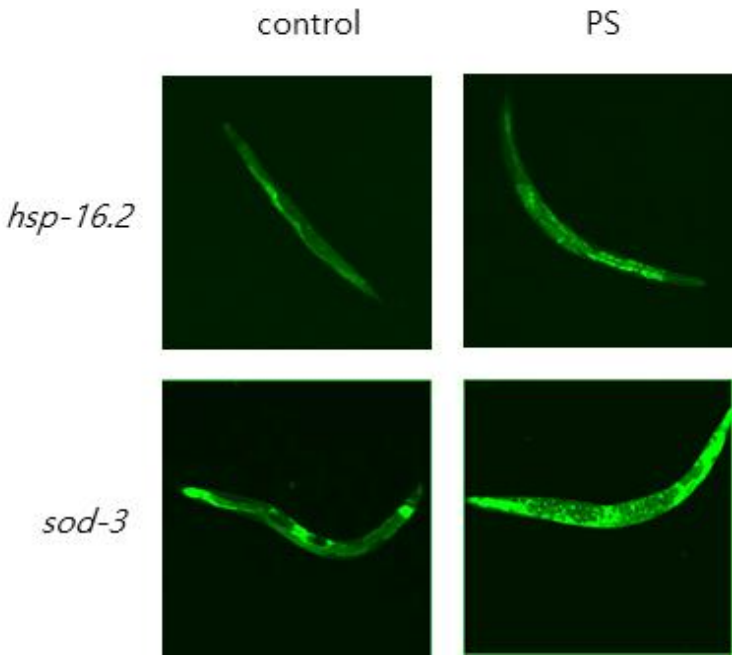

Figure 8. Induction of stress-responsive genes by phosphatidylserine. GFP expression of *hsp-16.2* and *sod-3* in untreated control and phosphatidylserine-treated group. PS, 100 ㎍/L of phosphatidylserine.

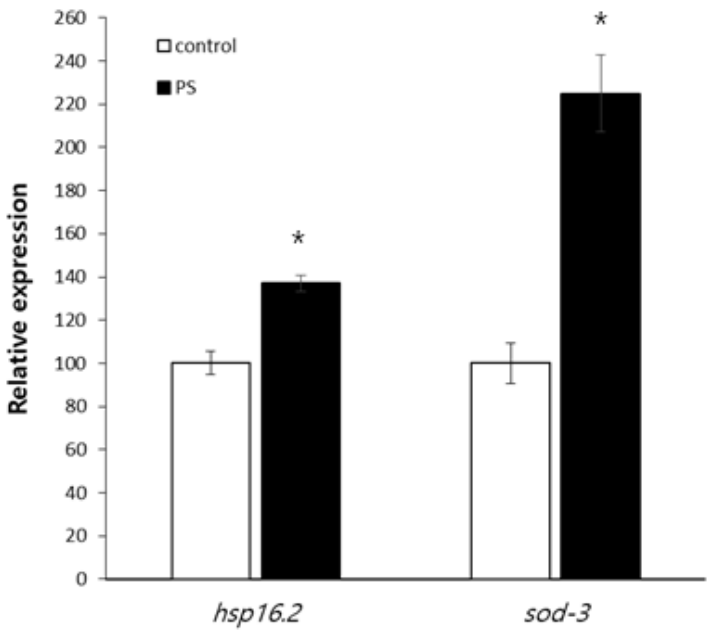

Figure 9. Quantification of GFP expression by stress-responsive genes. Quantification of GFP expression using the fluorescence intensity of untreated control set as 100. PS, 100 μg/L of phosphatidylserine; *, statistically significant ($P < 0.05$).

Ⅲ-7. Lifespan extension by phosphatidylserine overlapped with that of reduced insulin/IGF-1-like signaling.

In order to identify underlying mechanisms involved in phosphatidylserine induced longevity, we examined the effect of phosphatidylserine on lifespan of long-lived mutants. The long-lived mutants were *clk-1 (e2519)*, *eat-2 (ad465)* and *age-1 (hx546)*,. The *age-1 (hx546)* mutant has reduced insulin/IGF-1-like signaling and the *clk-1 (e2519)* mutant has a defect in mitochondrial electron transport chain reaction, resulting in reduced ROS production. The *eat-2 (ad465)* mutant causes reduced food pumping rate and is a widely-used genetic model of dietary restriction (DR). The lifespans of *clk-1 (e2519)* and *eat-2 (ad465)* mutants were significantly increased by phosphatidylserine (Fig. 10 and 11). Mean lifespan of *clk-1 (e2519)* increased from 18.1 days to 20.3 days ($P = 0.018$) and that of *eat-2 (ad465)* increased from 23.5 days 27.1 days ($P < 0.001$) by phosphatidylserine. However, the lifespan of *age-1 (hx546)* mutant was not altered by supplementation with phosphatidylserine (Fig. 12). These data indicate that the longevity phenotype conferred by phosphatidylserine overlaps with that by reduced insulin/IGF-1-like signaling. A repeated experiment showed the same effect of phosphatidylserine on three long-lived mutants (Table 2).

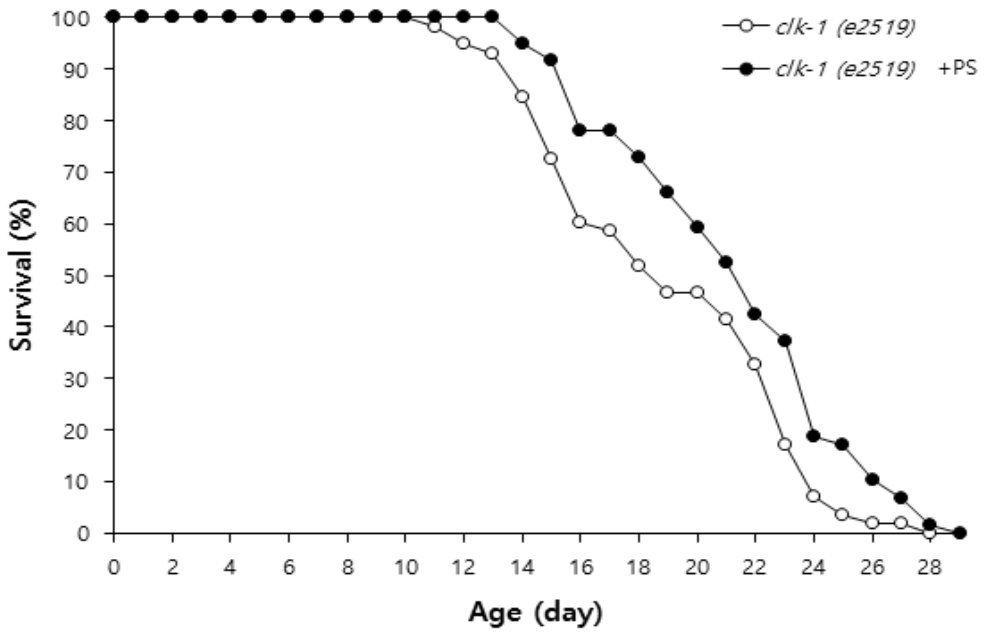

Figure 10. Effect of phosphatidylserine on lifespan of *clk-1 (e2519)*. Survival of *clk-1* mutants was compared betweem the untreated and phosphatidylserine-treated worms. PS, 100 ㎍/L of phosphatidylsrine.

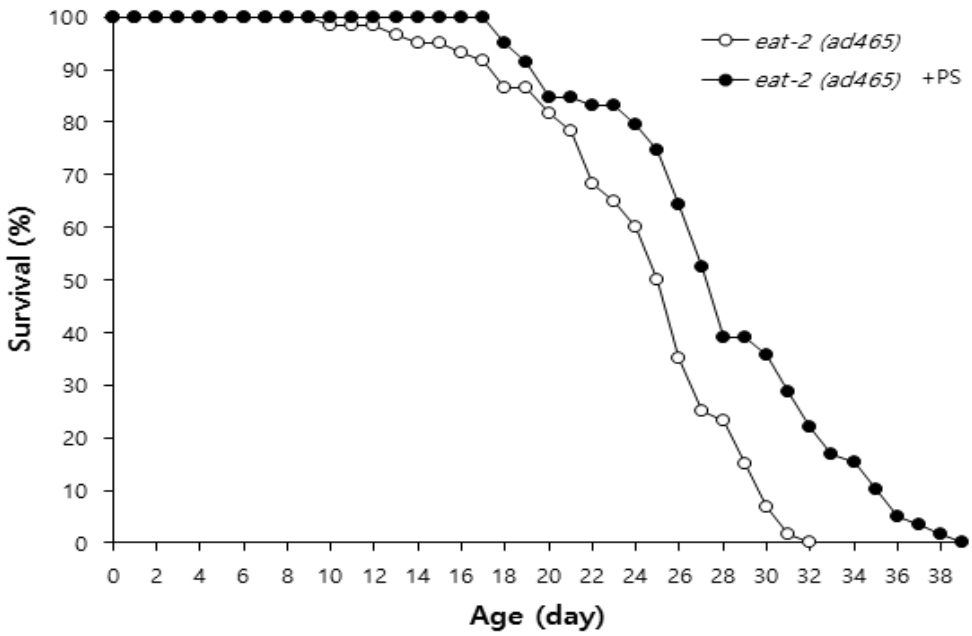

Figure 11. Effect of phosphatidylserine on lifespan of *eat-2 (ad465)*. Survival of *eat-2* mutants was compared betweem the untreated and phosphatidylserine-treated worms. PS, 100 ㎍/L of phosphatidylsrine.

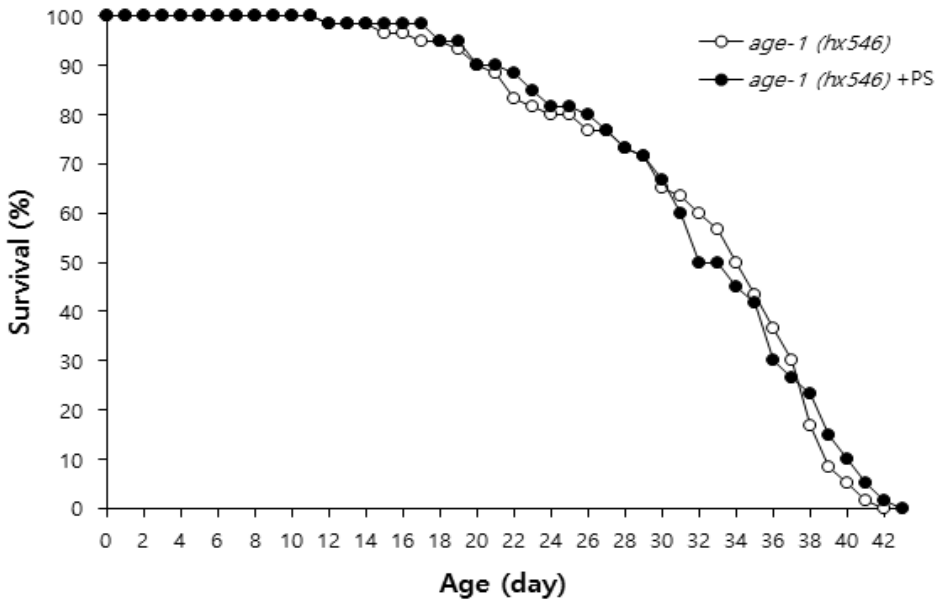

Figure 12. Effect of phosphatidylserine on lifespan of *age-1 (hx546)*. Survival of *age-1* mutants was compared betweem the untreated and phosphatidylserine-treated worms. PS, 100 ㎍/L of phosphatidylsrine.

Table 2. Effect of phosphatidylserine on lifespan of long-lived mutants.

		Mean lifespan (day)			
		Control	100 μg/l PS	*P* value[a]	% effect[b]
age-1 (*hx546*)	1st experiment	31.1	31.2	0.681	0.4
	2nd experiment	33.2	32.7	0.267	-1.4
clk-1 (*e2519*)	1st experiment	18.1	20.3	0.018	11.9
	2nd experiment	17.2	19.3	0.001	12.0
eat-2 (*ad465*)	1st experiment	23.5	27.1	< 0.001	15.3
	2nd experiment	24.7	26.5	0.001	7.6

[a]*P* value was calculated using the log-rank test by comparing the survival of the untreated control group (0 μg/L phosphatidylserine) to that of the phosphatidylserine-treated group (100 μg/L phosphatidylserine).

[b]% effects were calculated by $(C-S)/C*100$, where S is the mean lifespan of the phosphatidylserine-treated group and C is the mean lifespan of the untreated control group.

PS, phosphatidylserine.

Ⅲ-8. DAF-16 is required for the lifespan extension by phosphatidylserine.

The lifespan extension by mutations of genes involved in insulin/IGF-like signaling requires a downstream transcription factor DAF-16, regulating expression of various stress-responsive genes. We investigated the effect of *daf-16* knockdown on lifespan extension conferred by phosphatidylserine treatment. When the expression of *daf-16* was blocked using RNAi, extended lifespan by phosphatidylserine was completely disappeared (Fig. 13). Mean lifespan was increased from 17.6 days to 20.6 days ($P < 0.001$) by phosphatidylserine in empty vector control groups. On contrary, supplementation with phosphatidylserine failed to increase lifespan in worms treated with *daf-16* RNAi clone. In *daf-16* RNAi-treated group, mean lifespan was 17.0 days. In both *daf-16* RNAi- and phosphatidylserine-treated group, mean lifespan was 16.8 days ($P = 0.633$). These results show that DAF-16 is required for the lifespan extension by phosphatidylserine and supports our previous result that longevity phenotype by phosphatidylserine is mediated by reduced insulin/IGF-like-signaling.

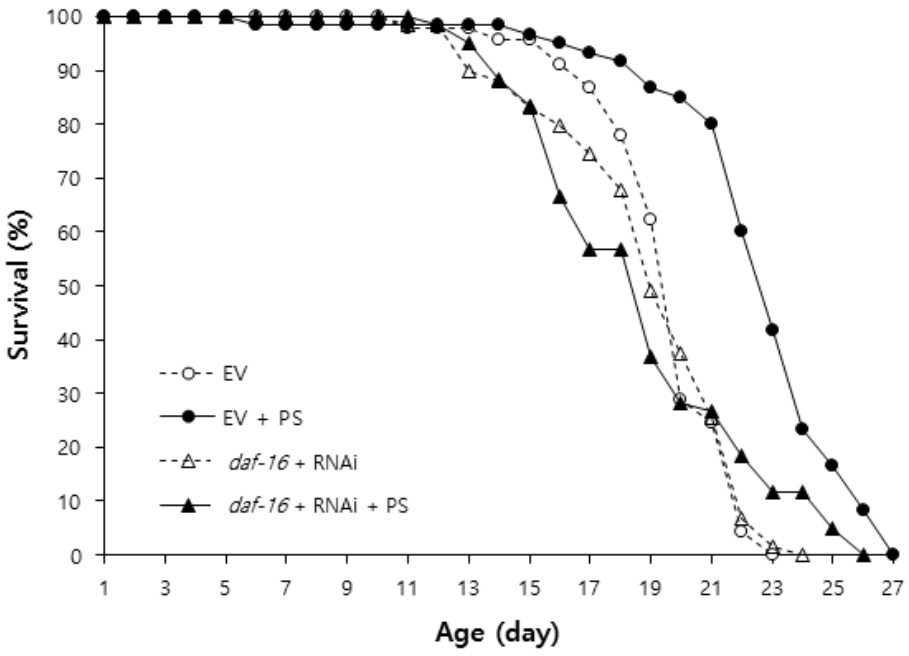

Figure 13. Role of DAF-16 on extended lifespan with phosphatidylserine. Requirement of DAF-16 for lifespan extension by phosphatidylserine was determined using gene knockdown by RNAi. PS, 100 μg/L of phosphatidylserine; EV, empty vector.

Ⅲ-9. Phosphatidylserine ameliorated Aβ-induced toxicity.

Next, we examined the effect of phosphatidylserine on age-related diseases. The CL4176 strain, in which human Aβ gene can be induced in muscle and cause paralysis, is a genetic model of AD. Phosphatidylserine-treated group significantly delayed paralysis induced human Aβ gene (Fig. 14). In the untreated group, the time of paralysis when 50% of worms were paralyzed was 3.8 h. Phosphatidylserine-treated group increased the time of 50% paralysis to 7.8 h, which was a 103.9% increase compared to the untreated control group ($P < 0.001$). Replicated experiments also showed a significant protective effect on Aβ-induced toxicity by supplementation with phosphatidylserine (Table 3.).

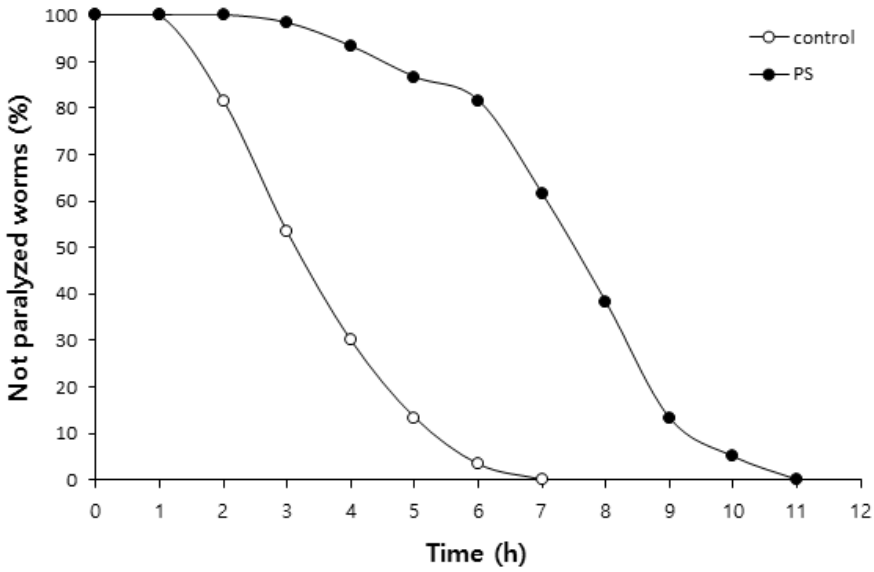

Figure 14. Effect of supplementation with phosphatidylserine on Aβ-induced toxicity. Paralysis caused by human Aβ transgene was compared between untreated control and phosphatidyslerine-treated group in AD model. PS, 100 ㎍/L of phosphatidylserine.

Table 3. Effect of phosphatidylserine on Aβ-induced toxicity in *C. elegans*.

	PS (μg/l)	Time when 50% of worms were paralyzed (h)	*P* value[a]	% effect[b]
1st experiment	0	3.8		
	100	7.8	< 0.001	103.9
2nd experiment	0	6.2		
	100	12.1	< 0.001	94.0
3rd experiment	0	5.2		
	100	13.4	< 0.001	156.7

[a]*P* value was calculated using the log-rank test by comparing the survival of the untreated control group (0 μg/L phosphatidylserine) to that of the phosphatidylserine-treated group (100 μg/L phosphatidylserine).

[b]% effects were calculated by $(C-S)/C*100$, where S is the time when 50% of worms were paralyzed in the phosphatidylserine-treated group and C is the time when 50% of worms were paralyzed in the untreated control group.

PS, phosphatidylserine.

Ⅲ-10. Reduced survival by HGD was recovered by phosphatidylserine.

Diabete mellitus (DM) is another well-known age-associated disease. In *C. elegans*, HGD leads to reduced survival and is used as a dietary model of DM. In this study, we showed that HGD decreased mean lifespan from 17.7 days in the untreated group to 15.6 days ($P < 0.001$) (Fig. 15). Interestingly, simultaneous treatment with HGD and phosphatidylserine restored reduced lifespan caused by HGD to normal. Mean lifespan of worms treated with both HGD and phosphatidylseirne was 17.1 days, which is significantly different from that of HGD-treated worms (15.6 days, $P = 0.001$). Independent replicative experiments showed a similar lifespan decrease by HGD and restoration of lifespan by phosphatidylserine (Table 4).

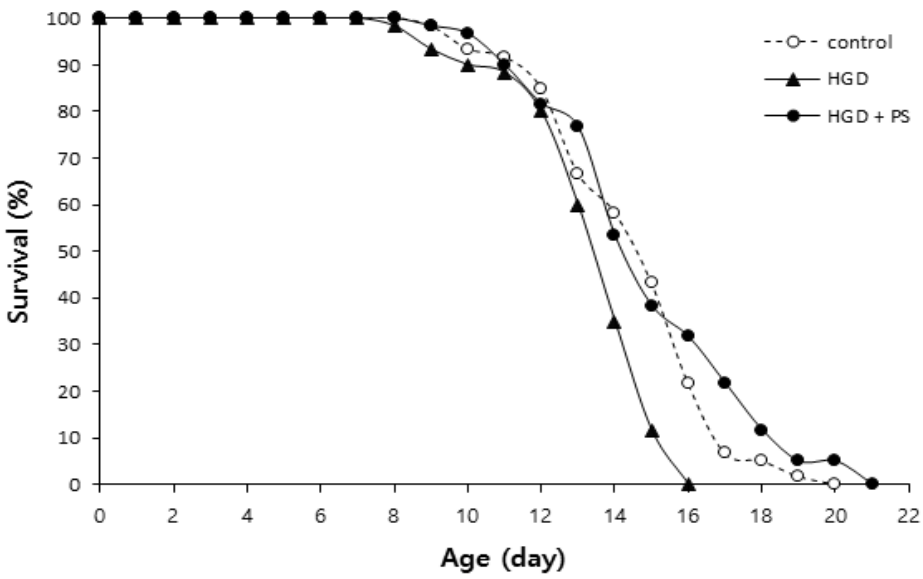

Figure 15. Effect of supplementation with phosphatidylserine on HGD-induced toxicity. Survival of worms with and without HGD were compared and the effect of phsopahtidylserine on the lifespan of worms treated with HGD was determined. PS, 100 100 μg/L of phosphatidylserine.

Table 4. Effect of high-glucose-diet and phosphatidylserine on lifespan of wild-type N2.

		Mean lifespan (day)	*P* value
1st experiment	N2	17.7	
	N2 + HGD	15.6	< 0.001[a]
	N2 + HGD + PS	17.1	0.001[b]
2nd experiment	N2	17.0	
	N2 + HGD	15.3	< 0.001[a]
	N2 + HGD + PS	16.2	0.025[b]
3rd experiment	N2	16.2	
	N2 + HGD	13.6	< 0.001[a]
	N2 + HGD + PS	14.6	0.005[b]

[a]*P* value was calculated using the log-rank test by comparing to the survival of N2.
[b]*P* value was calculated using the log-rank test by comparing to the survival of N2 + HGD.
HGD, high glucose diet (40 mM glucose); PS, phosphatidylserine.

Ⅳ. Discussion

Phosphatidylserine is a phospholipid found in biological membranes and rich in brain playing a key role in neuronal function (Lee et al. 2015; Abay et al. 2017). With aging, the amount of phosphatidylserine decreased (Suleimanova et al. 2017). In this study, we demonstrated anti-oxidant and anti-aging effect of phosphatidylserine for the first time *in vivo*. Dietary supplementation with phosphatidylserine increased resistance to oxidative stress and both mean and maximum lifespan. Based on the free radical theory of aging, which emphasizes the key role of oxidative damage in aging. Aging process was modulated by many anti-oxidants, including resveratrol, vitamin E, and curcumin (Harrington and Harley 1988; Gruber et al. 2007; Liao et al. 2011). Another theory of aging is the membrane theory, which suggests that decrease in membrane function with age leads to accumulation of toxic molecules (Pathath 2017). Solidification of membranes is also observed with aging, resulting decreased communication through membrane. Our previous study revealed that phosphatidylcholine showed anti-oxidant and anti-aging effects in *C. elegans* (Kim et al. 2019). Taken together, these results strongly suggest that improved membrane integrity by supplementation with phospholipids can retard aging process.

The disposable soma theory suggests that limited cellular resources should be allocated among cell maintenance, repair, and reproduction. Trade-off between increased lifespan and reduced fertility was observed in many lifespan-extending interventions. (Kirkwood 1977). The long-lived *age-1* mutant produced less progeny than the wild-type N2. Dietary supplementation with anti-oxidants, such as N-acetyl-L-cysteine, resveratrol, and phosphatidylcholine, extended lifespan and exhibited reduced fertility (Gruber et al. 2007; Oh et al. 2015; Kim et al. 2019). Treatment of phosphatidylserine also caused a reduction in fertility, which is a possible trade-off for long lifespan. One of biomarkers of aging conserved in many species is reduced motility.

Age-related decline of motility is associated with muscle dysfunction and atrophy (Del Campo, Jaimovich et al. 2016). Aged muscle is characterized by decreased muscle strength and mass, which are mainly caused by increased levels of ROS (Baumann et al. 2016). Supplementation of anti-oxidants, such as resveratrol and selenocysteine, have been reported to modulate muscular dysfunction with aging. Phosphatidylserine delayed decline in motility, which may be attributed to anti-oxidant activity. However, the underlying mechanisms of ROS in aged muscle has not been fully understood.

Based on increased resistance to oxidative stress and extended lifespan by phospahtidylserine, we expected decreased cellular of ROS level by dietary supplemenatation with phosphatidylserine. On contrary, cellular of ROS levels were significantly increased by phosphatidylserine treatment. These results suggest that phosphatidylserine may act as ROS generator, rather than ROS scavenger. Hormesis is defined as a "stimulatory effect of low doses of substances known to be toxic at higher doses" (Calabrese et al. 1999). Hormetic effects have been observed in various biological processes, including germination and growth, plant cell proliferation, and protein turnover (Calabrese et al. 1999). Previous studies reported that pre-treatment with low dose of stress, such as cold, heat and oxidative stress, increased resistance to the same stress and extended lifespan in *C. elegans* (Cypser et al. 2006). Hormesis-inducing stimulations cause pleiotropic amplification of effects including stress adaptation, increased survival, and reduced macromolecular damage (Rattan 2008). Hormetins are nutritional components inducing mild stress response and beneficial hormetic effects (Rattan 2008). Resveratrol showed dose-responsive hormetic effect (Lamming et al. 2004). In *C. elegans*, SOD-CAT mimetics induced oxidative stress response and increased lifespan (Melov et al. 2000). A well-known ROS generator, Juglone, up-regulation *hsp16.2* and induced hormesis (Hartwig et al. 2009). We observed that increased ROS led to increased resistance to oxidative stress and longevity by supplementation with phosphatidylserine. Therefore, it is sugggestive the

anti-oxidant and anti-aging effect of phosphatidylserine could be due to its hormetic effect. However, mechanism underlying hormesis is still unclear.

Genetic screening of long-lived mutants have identified several major lifespan-extending mechanisms in *C. elegans*. The first lifespan-extending mutation was found in *age-1*, which encodes an intracellular kinase mediating insulin/IGF-1-like signaling (Johnson 1990). The *age-1* mutations, reduced insulin/IGF-1-like signaling, conferred longevity phenotype. Other mutants, reduced insulin/IGF-1-like signaling, also resulted in lifespan extension (Kenyo et al. 1993). The other lifespan-extending mechanism is reduced mitochondrial function. Mutations in *clk-1*, a gene involved in biosynthesis of electron transport chain reaction component, ubiquinone, cause reduced ROS production (Wong et al. 1995). As a result, *clk-1* mutants show longevity phenotype. Genome-wide RNAi screening identified that defects in many genes involved in mitochondrial electron transport chain reaction confer a longevity phenotype in *C. elegans* (Dillin et al. 2002). DR is the well-known lifespan-extending intervention from yeast to monkey (Barger et al. 2003). The *eat-2* mutant was a genetic model of DR in *C. elegans*. *eat-2* mutants have reduced pharyngeal pumping rate and eat less than the wild-type control (Lakowski and Hekimi 1998). The lifespan assay with three long-lived mutants, *age-1, clk-1* and *eat-2*, revealed that lifespan extension by phosphatidylserine specifically overlapped with that by reduced insulin/IGF-1-like signaling. Knockdown of *daf-16*, a transcription factor working as a downstream effector of redcued inulin/IGF-1-like signaling, completely inhibited longevity phenotype conferred by phosphatidylseirne.

Many lifespan-extending interventions showed preventive effects on age-related diseases. DR in mice reduced incidence of cancer, cardiovascular diseases, neurodegenerative diseases (Sohal et al. 1994). DR in monkeys lowered blood pressure and plasma lipids and decreased development of type II diabetes and osteoarthritis(Colman et al. 2009). Previous studies showed that dietary supplementation with selenocysteine, N-acetyl - L-cysteine, or

phosphatidylcholine extended lifespan and significantly suppressed Aβ-induced toxicity in *C. elegans* (Oh and Park 2017; Kim et al. 2018, 2019). Anti-diabetic agents such as metformin and chicoric acid increased the lifespan of *C. elegans* and supplementation with selenocysteine reversed the increased mortality observed with HGD (Onken and Driscoll 2010; Schlernitzauer et al. 2013; Kim et al. 2018). To elucidate the effect of phosphatidylserine on age-related diseases, we used to genetic model of AD and nutritional models of DM. Dietary supplementation with phosphatidylserine was protective against Aβ-induced and HGD-induced toxicity. Our results suggest that phosphatidylserine has a preventive effect on age-related diseases in addition to lifespan-extending effect.

In conclusions, we showed anti-oxidant and anti-aging effects of phsophatidylserine in *C. elegans* and identified possible mechanisms involved, including hormesis and reduced inulin/IGF-1-like signaling. Supplementation with phosphatidylserine also showed preventive effects on age-related diseases. Further studies focusing on the genetic pathways involved in each bioactivities of phosphatidylserine and the effect of phosphatidylsernie in higher model organisms should be followed in a near future.

Ⅴ. References

Abay ZC, Wong MY, Teoh JS, Vijayaraghavan T, Hilliard MA, Neumann B (2017) Phosphatidylserine save-me signals drive functional recovery of severed axons in Caenorhabditis elegans. Proc Natl Acad Sci USA 114:E10196 - E10205

Abrahams S, Haylett WL, Johnson G, Carr JA, Bardien S (2019) Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: a review. Neuroscience 406:1 - 21

Adachi H, Ishii N (2000) Effects of tocotrienols on life span and protein carbonylation in *Caenorhabditis elegans*. J Gerontol A 55:B280 - 285

Barger JL, Walford RL, Weindruch R (2003) The retardation of aging by caloric restriction: its significance in the transgenic era. Exp Gerontol 38:1343 - 1351

Baumann CW, Kwak D, Liu HM, Thompson LV (2016) Ageinduced oxidative stress: how does it influence skeletal muscle quantity and quality? J Appl Physiol 121:1047 - 1052

Bayne AC, Sohal RS (2002) Effects of superoxide dismutase catalase mimetics on life span and oxidative stress resistance in the housefly, Musca domestica. Free Radic Biol Med 32:1229 - 1234

Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547 - 581

Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, Gems D (2011) Increased life span from overexpression of superoxide

dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. Free Radic Biol Med 51:1575 – 1582

Calabrese EJ, Baldwin LA, Holland CD (1999) Hormesis: a highly generalizable and reproducible phenomenon with important implications for risk assessment. Risk Anal 19:261 – 281

Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325:201 – 204

Cunnane SC, Schneider JA, Tangney C, Tremblay-Mercier J, Fortier M, Bennett DA, Morris MC (2012) Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 29:691 – 697

Cypser JR, Tedesco P, Johnson TE (2006) Hormesis and aging in *Caenorhabditis elegans*. Exp Gerontol 41:935 – 939

Deepa SS, Van Remmen H, Brooks SV, Faulkner JA, Larkin L, McArdle A, Jackson MJ, Vasilaki A, Richardson A (2019) Accelerated sarcopenia in Cu/Zn superoxide dismutase knockout mice. Free Radic Biol Med 132:19 – 23

Denham Harman (2001). Aging: overview, *Nebraska 68198-4635*
Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Rates of behavior and aging specified by mitochondrial function during development. Science 298:2398 – 2401

Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, Back P, Matscheski A, Vanfleteren JR, Gems D (2008) Against the oxidative damage

theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. Genes Dev 22:3236 - 3241

Ernst IM, Pallauf K, Bendall JK, Paulsen L, Nikolai S, Huebbe P, Roeder T, Rimbach G (2013) Vitamin E supplementation and lifespan in model organisms. Ageing Res Rev 12:365 - 375

Fukui K, Omoi NO, Hayasaka T, Shinnkai T, Suzuki S, Abe K, Urano S (2002) Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. Ann N Y Acad Sci 959:275 - 284

Gruber J, Tang SY, Halliwell B (2007) Evidence for a trade-off between survival and fitness caused by resveratrol treatment of *Caenorhabditis elegans*. Ann N Y Acad Sci 1100:530 - 542

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11:298 - 300

Harrington LA, Harley CB (1988) Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. Mech Ageing Dev 43:71 - 78

Hartwig K, Heidler T, Moch J, Daniel H, Wenzel U (2009) Feeding a ROS-generator to *Caenorhabditis elegans* leads to increased expression of small heat shock protein HSP- 16.2 and hormesis. Genes Nutr 4:59 - 67

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, SinclairDA (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425:191 - 196

Jackson JR, Ryan MJ, Always SE (2011) Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. J Gerontol A 66:751 - 764

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275:218 - 220

Jang YC, Lustgarten MS, Liu Y, Muller FL, Bhattacharya A, Liang H, Salmon AB, Brooks SV, Larkin L, Hayworth CR, Richardson A, Van Remmen H (2010) Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. FASEB J 24:1376 - 1390

Johnson TE (1990) Increased life-span of age-1 mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. Science 249:908 - 912

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421:231 - 237

Kunlin Jin, Modern Biological Theories of Aging (2010), Aging Dis 1(2): 72 - 74

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. Nature 366:461 - 464

Kim JS, Park SK (2017) Supplementation of S-allyl cysteine improves health

span in *Caenorhabditis elegans*. Biosci J 33:411 – 421

Kim JS, Kim SH, Park SK (2017) Selenocysteine modulates resistance to environmental stress and confers anti-aging effects in *C. elegans*. Clinics (Sao Paulo) 72:491 – 498

Kim SH, Kim BK, Park SK (2018) Selenocysteine mimics the effect of dietary restriction on lifespan via SKN1 and retards ageassociated pathophysiological changes in *Caenorhabditis elegans.* Mol Med Rep 18:5389 – 5398

Kim SH, Kim BK, Park S, Park SK (2019) Phosphatidylcholine extends lifespan via DAF-16 and reduces amyloid-beta induced *Caenorhabditis elegans.* toxicity inLongev 2019:2860642

Kirkwood TB (1977) Evolution of ageing. Nature 270:301 – 304

Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 95:13091 – 13096

Lamming DW, Wood JG, Sinclair DA (2004) Small molecules that regulate lifespan: evidence for xenohormesis. Mol Microbiol 53:1003 – 1009

Lee B, Sur BJ, Han JJ, Shim I, Her S, Lee YS, Lee HJ, Hahm DH (2015) Oral administration of squid lecithin-transphosphatidylated phosphatidylserine improves memory impairment in aged rats. Prog Neuropsychopharmacol Biol Psychiatry 56:1 – 10

Leventis PA, Grinstein S (2010) The distribution and function of phosphatidylserine in cellular membranes. Annu Rev Biophys 39:407 – 427

Liao VH, Yu CW, Chu YJ, Li WH, Hsieh YC, Wang TT (2011) Curcumin-mediated lifespan extension in *Caenorhabditis elegans*. Mech Ageing Dev 132:480 - 487

Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 21:8370 - 8377

Link CD (2006) *C. elegans* models of age-associated neurodegenerative diseases: lessons from transgenic worm models of Alzheimer's disease. Exp Gerontol 41:1007 - 1013

Linnane AW, Eastwood H (2006) Cellular redox regulation and prooxidant signaling systems: a new perspective on the free radical theory of aging. Ann N Y Acad Sci 1067:47 - 55

Long J, Gao H, Sun L, Liu J, Zhao-Wilson X (2009) Grape extract protects mitochondria from oxidative damage and improves locomotor dysfunction and extends lifespan in a *Drosophila* Parkinson's disease model. Rejuvenation Res 12:321 - 331

Marzetti E, Calvani R, Bernabei R, Leeuwenburgh C (2012) Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty—a mini-review. Gerontology 58:99 - 106

Mathieu Panel, Bijan Ghaleh, Didier Morin, Mitochondria and aging: A role for the mitochondrial transition pore?. Aging Cell 17(4):e12793

Mecocci P, Polidori MC, Troiano L, Cherubini A, Cecchetti R, Pini G, Straatman M, Monti D, Stahl W, Sies H, Franceschi C, Senin U (2000) Plasma

antioxidants and longevity: a study on healthy centenarians. Free Radic Biol Med 28:1243 – 1248

Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000) Extension of life-span with superoxide dismutase catalase mimetics. Science 289:1567 – 1569

Meng H, Kou J, Ma R, Ding W, Kou Y, Cao M, Dong Z, Bi Y, Thatte HS, Shi J (2017) Prognostic implications and procoagulant activity of phosphatidylserine exposure of blood cells and microparticles in patients with atrial fibrillation treated with pulmonary vein isolation. Mol Med Rep 16:8579 – 8588

Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, Huang TT, Epstein CJ, Roberts LJ 2nd, Csete M, Faulkner JA, Van Remmen H (2006) Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. Free Radic Biol Med 40:1993 – 2004

Naftelberg S, Ast G, Perlson E (2017) Phosphatidylserine improves axonal transport by inhibition of HDAC and has potential in treatment of neurodegenerative diseases. Neural Regen Res 12:534 – 537

Oh SI, Park SK (2017) N-acetyl-L-cysteine mimics the effect of dietary restriction on lifespan and reduces amyloid betainduced toxicity in *Caenorhabditis elegans*. Food Sci Biotechnol 26:783 – 790

Oh SI, Park JK, Park SK (2015) Lifespan extension and increased resistance to environmental stressors by N-acetyl-L-cysteine in *Caenorhabditis elegans*. Clinics (Sao Paulo) 70:380 – 386

Onken B, Driscoll M (2010) Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS ONE 5:e8758

Orr WC, Sohal RS (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science 263:1128 - 1130

Orr WC, Sohal RS (2003) Does overexpression of Cu, Zn-SOD extend life span in *Drosophila melanogaster*? Exp Gerontol 38:227 - 230

Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL (1998) Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nat Genet 19:171 - 174

Pathath AW (2017) Theories of aging. Inter J Indian Psychol 4:15 - 22

Perez VI, Bokov A, Van Remmen H, Mele J, Ran Q, Ikeno Y, Richardson A (2009a) Is the oxidative stress theory of aging dead? Biochim Biophys Acta 1790:1005 - 1014

Perez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A (2009b) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8:73 - 75

Peto R, Peto J (1972) Asymptotically efficient rank invariant test procedures. J R Stat Soc A 135:185 - 207

Pignolo RJ (2019) Exceptional Human Longevity. Mayo Clin Proc 94:110 - 124

Rattan SI (2008) Hormesis in aging. Ageing Res Rev 7:63 – 78 Rea SL, Wu D, Cypser JR, Vaupel JW, Johnson TE (2005) A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. Nat Genet 37:894 – 898

Rysavy NM, Shimoda LM, Dixon AM, Speck M, Stokes AJ, Turner H, Umemoto EY (2014) Beyond apoptosis: the mechanism and function of phosphatidylserine asymmetry in the membrane of activating mast cells. Bioarchitecture 4:127 – 137

Sanchez-Blanco A, Kim SK (2011) Variable pathogenicity determines individual lifespan in *Caenorhabditis elegans*. PLoS Genet 7:e1002047

Schlernitzauer A, Oiry C, Hamad R, Galas S, Cortade F, Chabi B, Casas F, Pessemesse L, Fouret G, Feillet-Coudray C, Cros G, Cabello G, Magous R, Wrutniak-Cabello C (2013) Chicoric acid is an antioxidant molecule that stimulates AMP kinase pathway in L6 myotubes and extends lifespan in *Caenorhabditis elegans*. PLoS ONE 8:e78788

Schlotterer A, Kukudov G, Bozorgmehr F, Hutter H, Du X, Oikonomou D, Ibrahim Y, Pfisterer F, Rabbani N, Thornalley P, Sayed A, Fleming T, Humpert P, Schwenger V, Zeier M, Hamann M, Stern D, Brownlee M, Bierhaus A, Nawroth P, Morcos M (2009) *C. elegans* as model for the study of high glucose-mediated life span reduction. Diabetes 58:2450 – 2456

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS (2005) Extension of murine life span by overexpression of catalase targeted to

mitochondria. Science 308:1909 – 1911

Seto NO, Hayashi S, Tener GM (1990) Overexpression of Cu-Zn superoxide dismutase in *Drosophila* does not affect lifespan. Proc Natl Acad Sci USA 87:4270 – 4274

Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. Mech Ageing Dev 74:121 – 133

Sohal RS, Agarwal A, Agarwal S, Orr WC (1995) Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age-related oxidative damage and increases metabolic potential in *Drosophila melanogaster*. J Biol Chem 270:15671 – 15674

Suleimanova RR, Hudz EA, Melnychuk DO, Kalachniuk LH (2017) Age-related changes phospholipids of sterlet in liver and dorsal muscles. Ukr Biochem J 89:71 – 75

Tan X, Shi J, Fu Y, Gao C, Yang X, Li J, Wang W, Hou J, Li H, Zhou J (2013) Role of erythrocytes and platelets in the hypercoagulable status in polycythemia vera through phosphatidylserine exposure and microparticle generation.

Wong A, Boutis P, Hekimi S (1995) Mutations in the clk-1 gene of *Caenorhabditis elegans* affect developmental and behavioral timing. Genetics 139:1247 – 1259

Xia N, Forstermann U, Li H (2014) Resveratrol as a gene regulator in the vasculature. Curr Pharm Biotechnol 15:401 – 408

Xia N, Daiber A, Forstermann U, Li H (2017) Antioxidant effects of resveratrol in the cardiovascular system. Br J Pharmacol 174:1633 - 1646

Ⅵ. 국문 요약

포스파티딜세린은 세포막에 존재하는 인지질 중 하나이며 뇌와 신경계에 풍부하게 존재한다. 포스파티딜세린의 함량은 노화가 진행됨에 따라서 감소하며, 이러한 감소는 노인의 인지 장애 및 알츠하이머병에 기여한다고 알려져 있다. 본 연구에서는 포스파티딜세린을 사용한 식이 보충이 예쁜꼬마선충의 생체 내 스트레스 반응 및 노화에 미치는 영향을 규명하였다. 포스파티딜세린 섭취는 산화성 스트레스에 대한 저항성을 유의적으로 증가시켰다. 산화성 스트레스는 노화 과정의 주원인이라고 알려져 있어, 다음으로 개체의 수명과 노화 관련 지표에 대한 영향을 확인하였다. 포스파티딜세린의 섭취는 개체의 평균 및 최대 수명을 유의적으로 증가시켰으며 수명연장의 부작용으로 알려진 번식력 감소를 동반하였다. 또한, 포스파티딜세린은 노화에 따른 운동성 감소를 지연시켰으며, 개체의 세포 내 활성산소량을 증가시킴과 동시에 스트레스 반응 유전자인 *hsp-16,2*와 *sod-3*의 발현을 유도하였다. 이러한 결과로 포스파티딜세린의 항산화 효능은 호르메시스 효과에 기인한다고 유추할 수 있다. 포스파티딜세린 섭취에 의한 수명연장 기전을 확인하기 위해 수명이 연장된 돌연변이들을 이용하여 분석해보았다. 그 결과, 포스파티딜세린에 의해 연장된 수명은 인슐린/IGF-1-like 신호 감소로 인한 수명연장과 중복되고 이때 전사조절인자 DAF-16이 필요하다는 것을 밝혔다. 노화 관련 질환에서의 효과를 규명하기 위해 대표적인 노화 관련 질환인 알츠하이머병과 당뇨병 동물모델을 이용하여 포스파티딜세린의 효과를 확인하였다. 포스파티딜세린은 예쁜꼬마선충의 알츠하이머병 모델에서 인간 아밀로이드 베타 유도 독성으로 인한 마비를 지연시켰다. 또한, 고 포도당 식이를 이용한 당뇨병 질환 모델에서 고 포도당 독성에 의해 감소한 생존율을 정상 수준으로 회복시켰다. 본 연구는 포스파티딜세린의 항산화 스트레스와 항노화 활성, 그리고 관련 기전을 최초로 규명하였다.