In: Proanthocyanidins
Editor: Ingrid Sullivan
ISBN: 978-1-63463-430-4

Chapter 6

ANTI-AGING EFFECTS OF APPLE PROCYANIDINS

Tadahiro Sunagawa[1,2], Isao Masuda[3,4] and Takahiko Shimizu[2,4*]

[1]Research Laboratories for Alcohol Beverages,
Asahi Breweries, Ltd., Ibaraki, Japan
[2]Molecular Gerontology, Tokyo Metropolitan Institute
of Gerontology, Tokyo, Japan
[3]Research & Development Laboratories for Innovation,
Asahi Group Holdings, Ltd., Ibaraki, Japan
[4]Department of Advanced Aging Medicine,
Chiba University Graduate School of Medicine, Chiba, Japan

ABSTRACT

Apple polyphenols (APs) mainly consist of procyanidins (PCs), which are composed of polymerized (–)-epicatechins and (+)-catechins. APs are contained in the edible part of the apple, so apples may be good source of dietary polyphenols. APs have been reported to display several beneficial health effects. In this chapter, we summarize the anti-aging effects of APs on age-related diseases in model organisms. First,

* Correspondence: Takahiko Shimizu, Ph.D.; Department of Advanced Aging Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan Telephone: +81-43-222-7171, Fax: +81-43-226-2095, E-mail: shimizut@chiba-u.jp

treatment with APs or PCs purified from APs extended the lifespan of *Caenorhabditis elegans* to a similar extent as treatment with *trans*-resveratrol. In contrast, treatment with APs had no effect on the longevity of *sir-2.1* worms, which lack the activity of SIR2, a member of the sirtuin family, indicating that APs showed SIR2-dependent anti-aging effects. In general, polyphenols have anti-oxidant activity. APs and PC dimers showed a higher level of superoxide dismutase-like activity and oxygen radical absorbance capacity than other polyphenols or antioxidants, as well as anti-oxidant effects on mouse myoblast cells *in vitro*. Next, we orally administered APs to heart/muscle-specific manganese-superoxide dismutase (Mn-SOD)-deficient mice, which exhibit lethal dilated cardiomyopathy due to intrinsic enhanced mitochondrial superoxide. Dietary APs significantly suppressed the progression of cardiac fibrosis, which extended the survival of the mutant mice. Dietary APs also suppressed oxidative DNA damage and reactive oxygen species production in enzymatically-dissociated cardiomyocytes of the mutant mice. Notably, dietary APs also improved the electrophysiological abnormalities, such as action potential and inwardly rectifier potassium current changes in the cardiomyocytes of the mutant mice, as determined using patch clamp techniques, and decreased the incidence of ventricular tachycardia or the induction of fibrillation using a burst ventricular pacing protocol. Furthermore, APs remarkably suppressed amyloid-β protein 42 (Aβ42) aggregation and dissociated Aβ42 aggregates *in vitro*, indicating that APs are potent suppressors of Aβ aggregation. APs also significantly inhibited Aβ neurotoxicity and stimulated the proliferation of neural PC-12 cells. Interestingly, treatment with APs also reduced polyglutamine aggregation in the *C. elegans* Q40 strain, a model of Huntington's disease. These findings strongly indicate that APs showed anti-aging effects, such as anti-oxidative, anti-protein aggregation and pro-longevity effects, using model organisms. Apple procyanidins are promising functional foods to prevent age-related disorders.

INTRODUCTION

Polyphenols include several groups of compounds, such as anthocyanins, flavonols and phenolic acids, which are accumulated in the plants themselves, as well as their fruits [1]. Several studies have reported that dietary polyphenols from plants prevent oxidative stress *in vivo* [2, 3]. Apples are one of the most common fruits grown and consumed worldwide, in addition to grapes, oranges and bananas [4]. The polyphenols extracted from apples (*Malus pumila* Mill., Rosaceae) mainly contain procyanidins (PCs), also called condensed tannins. PCs comprise approximately 65% of polyphenols from

unripe apples, and the remaining approximately 35% of compounds are regarded as the monomer fraction (MNs) (Figure 1A) [5]. PCs are formed by catechin oligomers composed of (−)-epicatechin and (+)-catechin monomers (Figure 1B) [6]. Procyanidin B1 (PB1) is an epicatechin-catechin dimer, procyanidin B2 (PB2) is an epicatechin-epicatechin dimer, and procyanidin C1 (PC1) is an epicatechin-epicatechin-epicatechin trimer. PCs are a kind of combination of high-molecular-mass polyphenols, the molecular mass distribution of which can be profiled using a size-exclusion HPLC method [7]. The profile of apple polyphenols (APs) is different from that of green tea (*Camellia sinensis*) polyphenols, which consist of monomer catechins, mainly epigallocatechin gallate (EGCG) (Figure 1C).

PCs are also contained in other fruits, such as grape (*Vitis* spp.) seeds [8], which are included in red wine, with the relationship between the health benefits and the PCs content of wine having been reported previously [9]. However, the profile of APs is different from that of grape seed polyphenols (Figure 1C), with apple PCs consisting of a larger amount of low-degree polymerized oligomers and a smaller amount of high-degree polymerized oligomers.

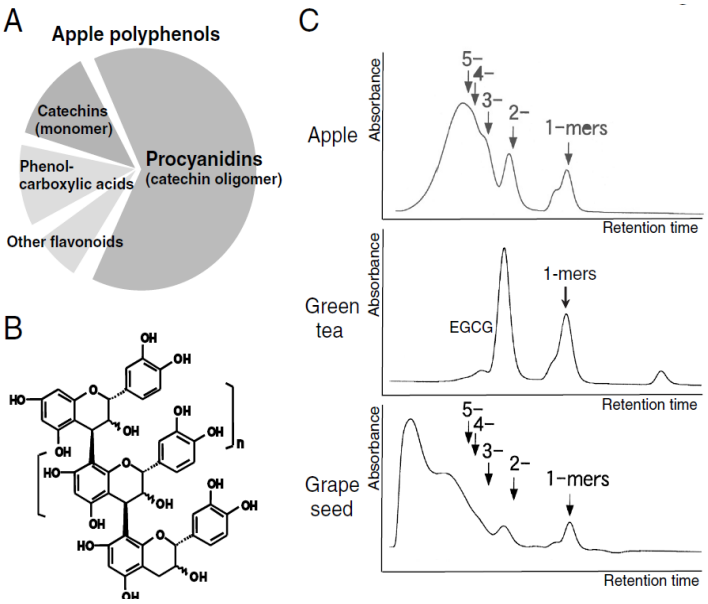

Figure 1. The composition of apple polyphenols (APs) (A), the chemical structure of apple procyanidins (PCs) (B) and the elution profiles of APs, tea polyphenols and grape seed polyphenols using gel chromatography (C) [7].

A structural analysis using NMR spectroscopy revealed that tetrameric PCs from unripe apples could be isolated, and the structures have been elucidated by NMR spectroscopy [10]. In addition, profiling of PC oligomer compounds from different apple varieties has been performing using comprehensive two-dimensional liquid chromatography [11]. The APs have been reported to exert various physiological functions, such as anti-tumor, anti-allergy and anti-obesity effects [12-14], as well as inhibitory effects on triglyceride adsorption, depending on the extent of polymerization of PCs in the human study [15]. However, the *in vivo* anti-aging effects, including the effects of the lifespan, redox balance and proteostasis, of APs and apple PCs have not been studied so far. In this chapter, we summarize the anti-aging effects of APs using model organisms for age-related diseases.

Apple Polyphenols Extend the Lifespan of *Caenorhabditis Elegans*

Caenorhabditis elegans is a simple multicellular organism with a short lifespan and generation time. Thus, *C. elegans* is widely used as an experimental model organism for studying aging and longevity. Dietary restriction extends the lifespan in *C. elegans*, as well as mammals [16]. We first evaluated the effects of APs and PCs purified from APs on the lifespan of *C. elegans*. Treatment with APs or PCs could significantly extend the mean lifespan of wild-type worms (Figure 2), to a similar extent as a *trans*-resveratrol [17]. In contrast, treatment groups given MNs purified from APs or epicatechin (the major component of the monomer fraction) did not show an extended lifespan [17], indicating that epicatechin oligomers, PCs, mainly contribute to the longevity effects of APs in worms. Wilson et al. have reported that blueberry extracts, which mainly contained PCs, also extended the lifespan of nematodes [18]. We thus concluded that PCs are a food factor regulating the lifespan of *C. elegans*.

It has been reported that *trans*-resveratrol extends the lifespan of several organisms in a SIR2-dependent manner [19, 20]. In order to investigate the involvement of SIR2 activity on the longevity effect of PCs in *C. elegans*, we examined the lifespan of *sir-2.1*worms, which are mutant worms that lack histone deacetylase (HDAC) SIR2 activity [19]. PCs could not extend the longevity of *sir-2.1* mutant worms, nor could *trans*-resveratrol. These findings indicated that PCs prolong the lifespan of worms in a SIR2-dependent manner. However, in an *in vitro* HDAC assay, PCs failed to activate SIRT1, in contrast

with *trans*-resveratrol, showing that the mechanism underlying the longevity effect is not due to a SIRT1 agonist effect. Since the upregulation of the *sir-2* gene extends the survival in worms [21], PCs might regulate *sir-2* expression.

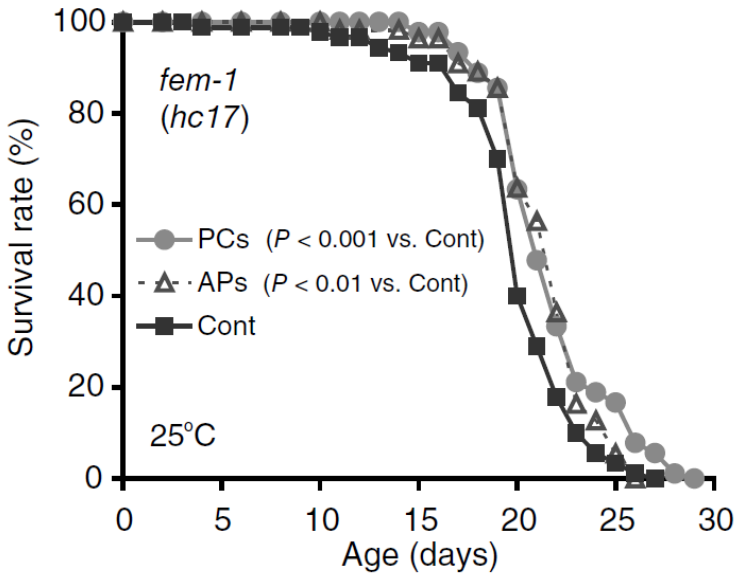

Figure 2. Apple PCs extend the lifespan of *Caenorhabditis elegans* [17]. The survival curves of *fem-1* worms treated with 65 μg/mL PCs (n = 90), 100 μg/mL APs (n = 55) and the control group (n = 89).

Apple Polyphenols Regulate Mitochondrial Superoxide Generation and Extend the Lifespan of a Mouse Model of Dilated Cardiomyopathy

A disturbance in the balance between the production of reactive oxygen species (ROS) and the antioxidant defense system causes oxidative stress, which leads to tissue injury and dysfunction [22,23]. It has been proposed that the accumulation of oxidative damage accelerates aging [24]. Moreover, in the heart, the generation of ROS and the age-related loss of antioxidant capacity might be involved in age-related cardiac dysfunction [25-27].

In order to examine the anti-oxidative effects of APs *in vitro*, we measured the superoxide dismutase (SOD)-like activity [28] and oxygen radical absorbance capacity (ORAC) [29] of APs and PCs. The SOD-like

activity of APs, PC dimers, PB1 and PB2, and PC trimer, PC1, was higher than that of EUK-134, a mimetic of SOD (Figure 3A). In particular, PB1 and PB2 showed a high level of SOD-like activity. APs, PB1, PB2, and PC1, as well as *trans*-resveratrol, also showed a high level of ORAC compared to the antioxidant vitamin C or EGCG (Figure 3B).

In order to examine the anti-oxidative effects of APs *in vitro*, we then measured the intracellular ROS levels of C2C12 cells that had been treated with H_2O_2 using the fluorescent probe.

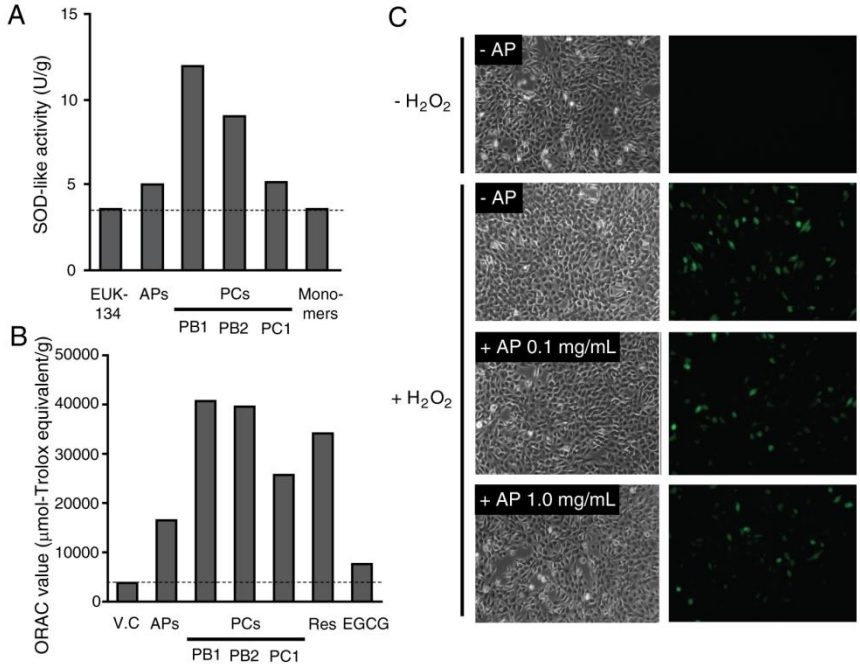

Figure 3. APs and apple PCs have anti-oxidant activity. The superoxide dismutase (SOD)-like activity of polyphenols (A), and the oxygen radical absorbance capacity (ORAC) values of polyphenols (B). The SOD-like activity was determined using the cellular xanthine/xanthine oxidase system as a superoxide source [28]. The ORAC assay was assessed according to the method described by Ishimoto et al. [29]. The 2,2'-azobis(2-amidinopropane)dihydrochloride was used as a peroxyl radical generator, and 6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acids (Trolox) was used as a standard. Monomers: monomer fraction of APs, V.C: vitamin C, Res: *trans*-resveratrol, EGCG: epigallocatechin gallate. The anti-oxidative effects of APs on mouse C2C12 myoblast cells (C) [30]. The cells were preincubated with APs and then were incubated with H_2O_2 and then with CM-H_2DCFDA. ROS production was induced in the cells treated with H_2O_2, as assessed by DCF fluorescence.

As a result, we found that preincubation with APs suppressed the production of ROS in C2C12 cells treated with H_2O_2 in a dose-dependent manner (Figure 3C). Next, in order to examine the anti-oxidative effects of APs against intrinsic ROS, we measured the intracellular ROS level of tamoxifen-induced manganese-superoxide dismutase (Mn-SOD) deficient mouse embryonic fibroblast (MEF) cells using the fluorescent probe. These studies showed that preincubation with APs suppressed the production of ROS in Mn-SOD-deficient MEFs [30].

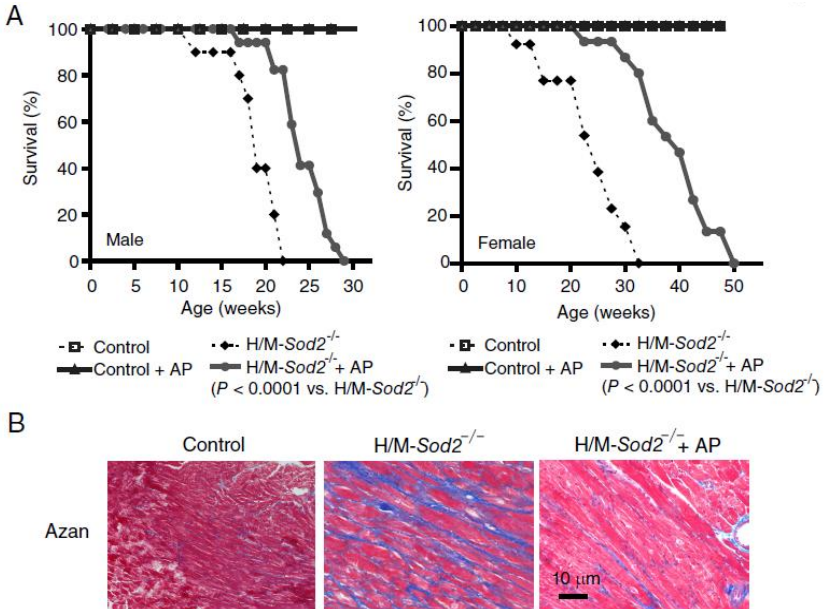

Figure 4. APs improve the survival and myocardial degeneration of H/M-*Sod2*$^{-/-}$ mice [30]. The survival curves of male and female H/M-*Sod2*$^{-/-}$ mice (A). The intake of APs extended the mean lifespan of the mice by 29% and 72% compared with water alone. Male H/M-*Sod2*$^{-/-}$; n = 10, H/M-*Sod2*$^{-/-}$+AP; n = 17, female H/M-*Sod2*$^{-/-}$; n = 13, H/M-*Sod2*$^{-/-}$+AP; n = 15. Azan-stained transverse sections of the left ventricular walls (B). The LV walls from male H/M-*Sod2*$^{-/-}$ mice showed myocardial degeneration, cardiomyocyte disarray and diffuse myocardial fibrosis. The intake of APs ameliorated the symptoms of the mutant mice.

Mn-SOD is an antioxidant enzyme that is located in mitochondria and catalyzes the dismutation of superoxide into oxygen and H_2O_2. We previously reported that conditional knockout mice that specifically lacked Mn-SOD in their cardiac and skeletal muscle developed progressive dilated cardiomyopathy (DCM) [31]. When APs were administered to heart and

muscle Mn-SOD-deficient (H/M-*Sod2*$^{-/-}$) mice in drinking water containing 0.1% (w/v) APs, the APs significantly extended the lifespan of the male and female mutant mice (Figure 4A).

Dietary APs also significantly delayed their progressive body weight loss and cardiac dilatation. Moreover, APs treatment mitigated the accumulation of plasma creatine phosphokinase (CPK), the histopathology and the fibrosis in the heart of the mutant mice (Figure 4B). The biochemical analyses performed using ROS-reacting fluorescent reagents revealed that APs supplementation reduced the ROS production in the cardiomyocytes of mutant hearts *in vivo* [30]. Furthermore, dietary APs tended to decrease the 8-oxodG level of the nuclear fractions of the hearts of the H/M-*Sod2*$^{-/-}$ mice [30]. It is noteworthy that the concentration of APs in drinking water was 0.1% (w/v), which is equivalent to the level present in apple juice [32]. We previously reported that the administration of an SOD/catalase mimetic, EUK-8, significantly ameliorated the cardiac pathology and dysfunction of H/M-*Sod2*$^{-/-}$ mice by suppressing mitochondrial oxidative stress [33]. Taken together with our results, these data indicate that APs suppress mitochondrial superoxide production via their anti-oxidative effects, and prevent oxidative stress.

Apple Polyphenols Prevent Cardiac Electrophysiological Abnormalities of a High-Frequency Mouse Model of Ventricular Arrhythmias

Cardiac arrhythmia is a major health problem in the elderly. Although the incidence of ventricular arrhythmias is higher in patients with heart disease, premature ventricular contractions (PVCs) and multiform PVCs are common even in healthy aged people [34-36]. Oxidative stress participates in the age-related changes in the electromechanical function of the heart [25, 37, 38]. The surface electrocardiogram (ECG) showed that there was a significant prolongation in the QRS, PR and QT intervals and a flattening of the J wave observed in H/M-*Sod2*$^{-/-}$ mice [39]. The prolonged QT interval might stem from delayed repolarization of the ventricular action potentials and the increases in the duration of the QRS complex interval might reflect slowed conduction in mice. Dietary APs partially reversed the changes in the QRS interval in the ECG parameters of H/M-*Sod2*$^{-/-}$ mice, although it did not affect the ECG parameters of control mice [39].

The effective refractive period (ERP) from the left ventricle was significantly prolonged in the hearts of H/M-*Sod2*$^{-/-}$ mice relative to that of

control mice [39], indicating that chronic exposure to oxidative stress affects the repolarization phase of the cardiac action potential. To determine whether chronic exposure to oxidative stress increases the susceptibility to ventricular arrhythmias, we also examined the induction of ventricular tachycardia (VT) or ventricular fibrillation (VF) using a burst ventricular pacing protocol (Figure 5A). In control mice, burst pacing failed to induce VT or VF, regardless of whether the mice received APs. In contrast, VT or VF could be induced in the H/M-*Sod2*$^{-/-}$ mice (Figure 5B). In APs-treated H/M-*Sod2*$^{-/-}$ mice, the ERP and incidence of VT or VF induction were significantly decreased, indicating that dietary APs improve the electrophysiological abnormalities arising due to chronic exposure to ROS.

Using whole-cell patch-clamp techniques with enzymatically-dissociated myocytes (Figure 5C), the membrane capacitance (Cm), which was used as an indirect index of the cell size, for the left ventricular myocytes of H/M-*Sod2*$^{-/-}$ mice was significantly greater than that of control mice, and dietary APs significantly reduced the Cm value of H/M-*Sod2*$^{-/-}$ myocytes [39]. The action potential durations (APD) at a 50% or 90% repolarization level in the left ventricular myocytes of H/M-*Sod2*$^{-/-}$ mice were significantly longer than those of control myocytes (Figure 5D). Dietary APs significantly reversed the prolongation of the APD in the left ventricular myocytes of H/M-*Sod2*$^{-/-}$ mice, although it did not influence the APDs in the control mice [39]. Abnormal automaticity, such as early afterdepolarizations, probably resulting from incomplete repolarization, was observed in about half of the ventricular cells of H/M-*Sod2*$^{-/-}$ mice, and dietary APs significantly inhibited the appearance of abnormal automaticity in H/M-*Sod2*$^{-/-}$ myocytes [39]. The dispersion of prolonged APDs, as well as the abnormal automaticity in the ventricular myocardium, might contribute to the increased susceptibility of H/M-*Sod2*$^{-/-}$ hearts to VT or VF.

There were no significant differences in the densities of whole-cell membrane currents, such as L-type Ca^{2+} or outward K^+ in the left ventricular myocytes isolated from H/M-*Sod2*$^{-/-}$ mice. In contrast, the density of the inwardly rectifier K^+ current (I_{K1}) was significantly lower in the left ventricular myocytes of H/M-*Sod2*$^{-/-}$ mice than in control mice, and dietary APs significantly increased the outward component of the I_{K1} at -50 mV in H/M-*Sod2*$^{-/-}$ ventricular cells, although it did not increase the density of I_{K1} in the ventricular cells of control mice (Figure 5E).

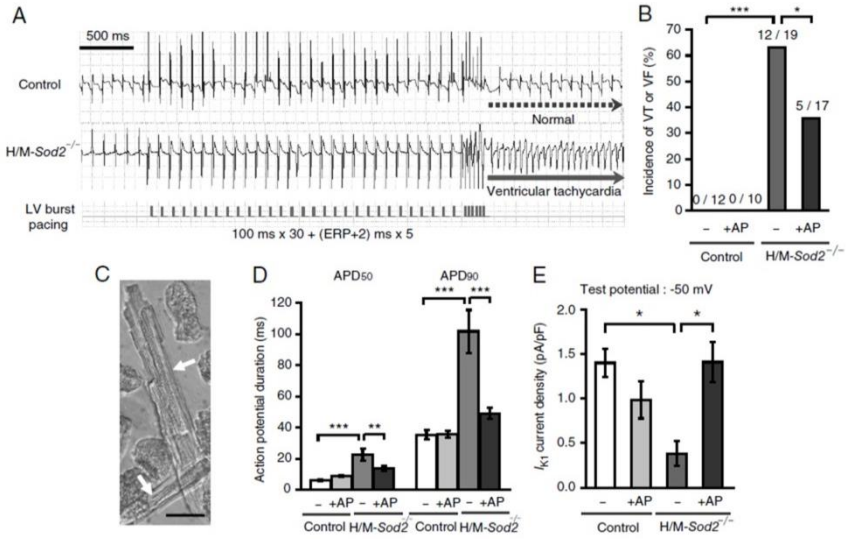

Figure 5. APs improve the cardiac electrophysiological abnormalities of H/M-*Sod2*$^{-/-}$ mice [39]. Representative ECG records during burst ventricular pacing observed in a control mouse and a H/M-*Sod2*$^{-/-}$ mouse (A). The incidence of ventricular tachycardia (VT) or ventricular fibrillation (VF) induced by burst pacing in control and H/M-*Sod2*$^{-/-}$ mice with and without AP treatment (B). Dietary APs decreased the incidence of VT or VF induction in H/M-*Sod2*$^{-/-}$ mice. Differences between groups, ***$P < 0.001$, *$P < 0.05$. The microscopic observation of enzymatically-dissociated left ventricular cardiomyocytes from H/M-*Sod2*$^{-/-}$ mice (C). The arrows show cardiomyocytes. The bar shows 20 μm. The action potential durations at 50% and 90% repolarization levels (APD50 and APD90) in the myocytes in each group (D). Control, $n = 49$; Control+AP, $n = 32$; H/M-*Sod2*$^{-/-}$, $n = 32$; and H/M-*Sod2*$^{-/-}$+AP, $n = 41$. ***$P <$ 0.001, **$P < 0.01$. The density of the I_{K1} in H/M-*Sod2*$^{-/-}$ ventricular cells (E). The values are the means ± SEM, Control, $n = 32$; Control + AP, $n = 31$; H/M-*Sod2*$^{-/-}$, $n =$ 32; and H/M-*Sod2*$^{-/-}$ + AP, $n = 32$. Differences between groups, *$P < 0.05$.

The inward rectifier K^+ channel provides one of the major components of the repolarizing outward current, the I_{K1}, in the cardiac action potential [40, 41]. The reduction in the I_{K1} may be involved in the prolongation of APDs, as well as the appearance of abnormal automaticity. Several studies demonstrated a significant suppression of the inwardly rectified K^+ channel by acute exposure to oxidative stress [42-44]. In the hearts of the Mn-SOD deficient mice, excess ROS reduced the I_{K1}, and dietary APs might prevent cardiac electrophysiological abnormalities due to a recovery of the I_{K1} as a result of their anti-oxidative effects.

Apple Polyphenols Suppress Protein Aggregation

Alzheimer's disease (AD) is a typical age-related and progressive neurodegenerative disease with memory impairment in later life. AD is diagnosed by the presence of amyloid accumulation, which is observed as a deposition in the hippocampus and cerebral cortex, frequently referred to as "senile plaque" [45]. Amyloid β-protein 42 (Aβ42), which consists of 42 residues, is observed mainly in the core of senile plaques. The protein forms strong self-aggregates that are associated with neurotoxicity *in vitro* [46]. In an Aβ42-thioflavin-T (Th-T) binding assay, APs and PCs dramatically abrogated the Aβ aggregation [47]. PCs inhibited the Aβ aggregation in a dose-dependent manner at an IC50 of 4.8 μg/mL. Aggregates were decreased by high-dose APs or PCs. PCs had approximately two-fold the anti-Aβ aggregative ability compared to APs. In contrast, MNs showed limited effects on Aβ aggregation.

We next confirmed the inhibitory effects of PCs on amyloid aggregation using a centrifugation method, to exclude the possibility that exogenous compounds affected the Th-T fluorescence intensity [48]. We observed the typical aggregates of Aβ after incubation (Figure 6A; vehicle). Treatment with 100 μg/mL APs and 65 μg/mL PCs completely diminished the aggregates (Figure 6A; APs and PCs), while 35 μg/mL MNs treatment did not (data not shown), demonstrating that PCs and APs exhibited strong anti-amyloidogenic ability *in vitro*.

PCs also dissociated Aβ42 aggregates following a post-treatment with polyphenols [47]. Although APs and PCs are orange-brown in color, we observed a brown-colored pellet that formed following treatment of Aβ42 aggregates with PCs and APs, suggesting that PCs could bind to Aβ42 aggregates. On the other hand, treatments with either vitamin-C or the potent antioxidative agent, EUK-134, did not suppress the Aβ aggregation, thus suggesting that the anti-aggregative activity of PCs is likely independent of its antioxidant properties. The chemical structures of these compounds might affect the Aβ aggregation, rather than their antioxidative capacity.

Aβ42 plays a pivotal role in the pathogenesis AD because of its potent aggregative ability and neurotoxicity [49]. PCs also had neuroprotective effects against Aβ42-induced neurotoxicity of neural PC-12 cells, as determined using a MTT assay (Figure 6B) [50]. PCs have a more potent ability to promote Aβ disaggregation and neuroprotection than whole APs. In contrast, MNs showed limited depression of Aβ aggregation.

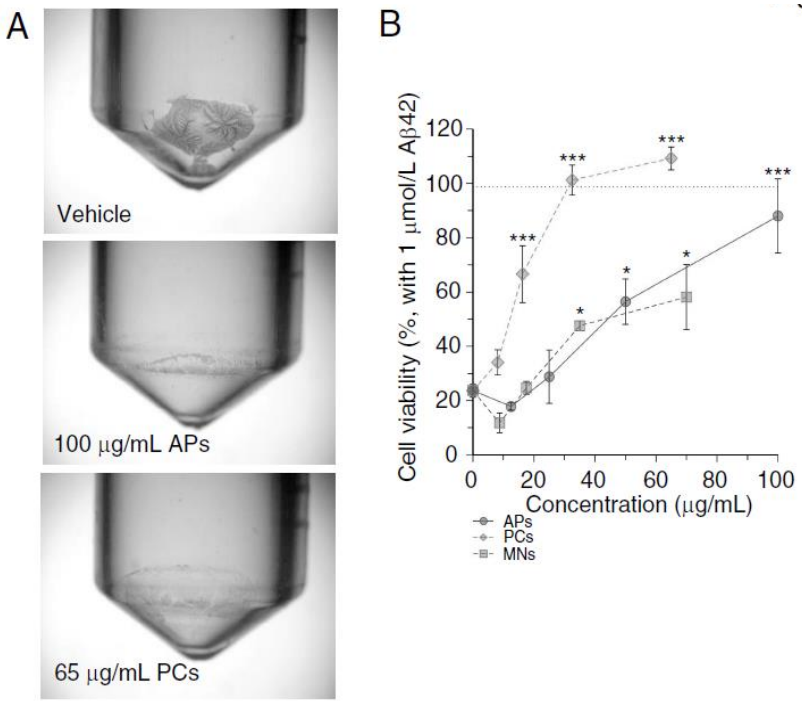

Figure 6. Apple PCs suppress and extinguish Aβ aggregation *in vitro* [47]. Microscopic observations of Aβ42 precipitates by centrifugation in the presence of polyphenols (A). The viability of PC-12 cells treated with Aβ42 (B). Cells were pretreated with various concentrations of APs, PCs and MNs, and then were incubated with Aβ42. PCs restored the viability of PC-12 cells treated with Aβ42. The values are the means ± SEM, $n = 3$. Compared to the control group, ***$P < 0.001$, and *$P < 0.05$.

It is possible that whole APs and MNs may contain factors that counteract the anti-aggregative and neuroprotective activity of PCs. Grape seed polyphenolic extracts including PCs interfere with paired helical filament formation by direct physical intercalation with taumolecules [51]. Therefore, apple PCs might have a similar suppressive effect on intermolecular aggregation of tau, as well as Aβ42.

Huntington's disease (HD) is also a progressive neurodegenerative disease, and is diagnosed by the presence of polyglutamine (polyQ) aggregation observed in addition to the atrophy of the cerebral cortex or basal ganglion [52]. Interestingly, PCs treatment also reduced polyQ deposition in body wall muscle cells of the transgenic *C. elegans* Q40 strain, a model of HD (Figure 7A, B) [53], indicating that apple PCs inhibited endogenous polyQ aggregation *in vivo*.

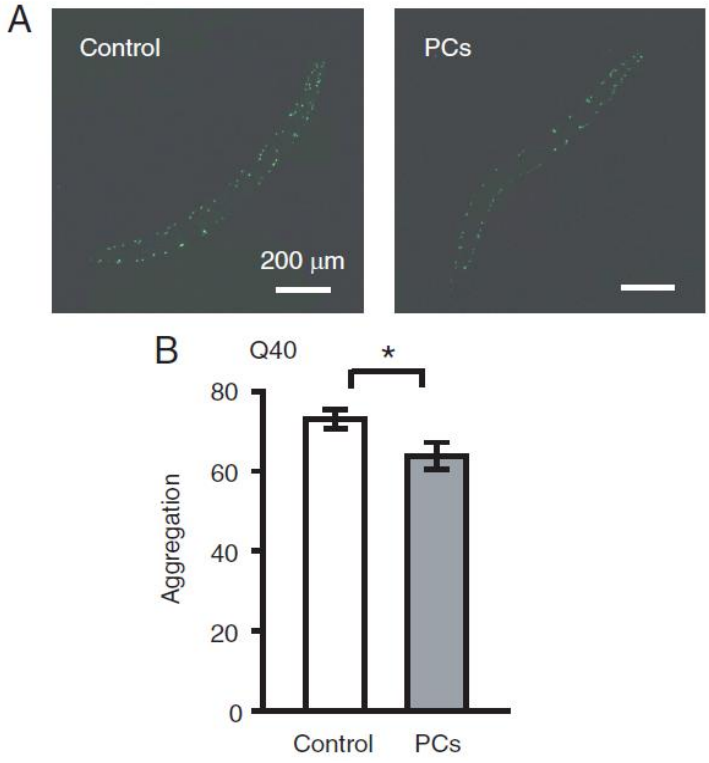

Figure 7. Apple PCs reduce the polyglutamine (polyQ) aggregation in *C. elegans*. The *C. elegans* Q40 strain was treated with PCs (A). Q40-YFP deposits were observed as dots. The number of aggregates of Q40 worms treated with PCs (12-day-old) (B). PCs reduced the aggregate formation in the worms. The values are the means ± SEM, control; $n = 18$, PCs; $n = 9$. Compared to the control group, $^*P < 0.05$.

Apple Polyphenols Are Good Food Factors to Prevent Age-Related Disorders

The current research shows that both nutritional apple- and pharmaceutical statin-based approaches may have the potential to reduce mortality due to vascular disease, recalling the phrase that "An apple a day keeps the doctor away" [54]. Recently, Caton et al. report that apple PCs showed beneficial effects on vascular endothelial function [55], as well as an inhibitory effect on lectin-like oxidized LDL receptor-1, which is a key player in the development of atherosclerosis [56]. It also showed activating effects on K^+ channels of aortic endothelial cells [57], and anti-inflammatory effects on intestinal

epithelial cells [58]. The tetrameric PC increases the levels of a gastrointestinal hormone, GLP-1, and insulin secretion in mice, showing that PCs act as agonists in the gut [59]. Further studies are necessary to elucidate the influence of the degree of PCs polymerization on their favorable effects.

This chapter showed that APs and apple PCs have several anti-aging effects on organisms *in vitro* and *in vivo* (Figure 8). APs had a potent *in vivo* cardioprotective effect in a mouse model of DCM and ventricular arrhythmia, and extended the lifespan of the mice. APs mainly contain PCs, which have potent antioxidative activity [60]. The bioavailability of APs has been reported previously [61]. Shoji et al. revealed that apple PCs, which range in length from dimers to pentamers, are detectable in rat plasma after their oral administration. Since the ROS levels were lower in the ventricular cells of dietary APs 0.1% (w/v) water-treated H/M-*Sod2*$^{-/-}$ mice than in untreated H/M-*Sod2*$^{-/-}$ mice, the favorable cardioprotective effects might be ascribed to the antioxidant effects of APs.

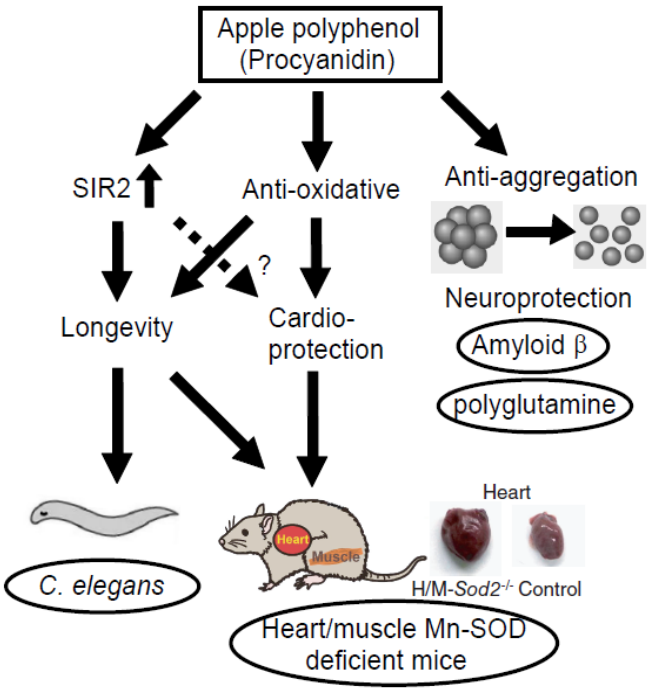

Figure 8. The APs and apple PCs have several anti-aging effects on organisms *in vitro* and *in vivo*.

In this chapter, we also revealed that APs showed SIR2-dependent anti-aging effects leading to lifespan extension in a nematode. SIRT1 may have an important role in the ability of the compounds to protect against cardiac ischemia [62]. It has been demonstrated that APs reduce the oxidative stress and inflammation involved in inflammatory bowel diseases, and these actions were accompanied by the induction of Nrf2 (orchestrating cellular antioxidant defenses and maintaining redox homeostasis) [63]. Taken together, these finding led us to propose that the beneficial effects of APs on lifespan involve not only a direct radical scavenging action, but also the modulation of activity of sirtuins, and/or an indirect anti-oxidative effect via the upregulation of cellular defense systems in cardiomyocytes. Further studies are necessary to elucidate the molecular mechanisms responsible for the effects of APs.

Apples are one of the major fruits cultivated and consumed all over the world. APs are contained in the edible part of the apple, and so are easier to routinely ingest compared to other active polyphenols, such as *trans*-resveratrol and grape seed polyphenols [64, 65]. Apple polyphenols and apple procyanidins may be promising and potential functional food factors to prevent or treat age-related disorders.

CONCLUSION

This chapter shows that apple polyphenols mainly consist of procyanidins have the *in vitro* and *in vivo* anti-aging effects, including the effects of the lifespan, redox balance and proteostasis. We propose that the beneficial effects on the lifespan involve not only a direct radical scavenging action, but also the modulation of activity of sirtuins, and/or an indirect anti-oxidative effect. Apple polyphenols may be promising and potential functional food factors to prevent or treat age-related disorders.

REFERENCES

[1] Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2, 1231-1246.

[2] Pietta, P. G. (2000). Flavonoids as antioxidants. *J. Nat. Prod.*, 63, 1035-1042.

[3] Mursu, J., Voutilainen, S., Nurmi, T., Tuomainen, T. P., Kurl, S. & Salonen, J. T. (2008). Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-aged Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Br. J. Nutr.*, 100, 890-895.

[4] Food and Agriculture Organization of the United Nations, FAOSTAT database (FAOSTAT, 2012). Available at http://faostat.fao.org/.

[5] Shoji, T., Masumoto, S., Moriichi, N., Kanda, T. & Ohtake, Y. (2006). Apple (*Malus pumila*) procyanidins fractionated according to the degree of polymerization using normal-phase chromatography and characterized by HPLC-ESI/MS and MALDI-TOF/MS. *J. Chromatogr.* A, 1102, 206-213.

[6] Ohnishi-Kameyama, M., Yanagida, A., Kanda, T. & Nagata, T. (1997). Identification of catechin oligomers from apple (*Malus pumila* cv. Fuji) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry. *Rapid Commun. Mass Spectrom.*, 11, 31-36.

[7] Yanagida, A., Shoji, T. & Kanda, T. (2002). Characterization of polymerized polyphenols by size-exclusion HPLC. *Biosci. Biotechnol. Biochem.*, 66, 1972-1975.

[8] Santos-Buelga, C. & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds: Nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.*, 80, 1094-1117.

[9] Corder, R., Mullen, W., Khan, N. Q., Marks, S. C., Wood, E. G., Carrier, M. J. & Crozier, A. (2006). Oenology: red wine procyanidins and vascular health. *Nature*, 444, 566.

[10] Nakashima, S., Oda, C., Masuda, S., Tagashira, M. & Kanda, T. (2012). Isolation and structure elucidation of tetrameric procyanidins from unripe apples (*Malus pumila* cv. Fuji) by NMR spectroscopy. *Phytochemistry*, 83, 144-152.

[11] Montero, L., Herrero, M., Ibáñez, E. & Cifuentes, A. (2013). Profiling of phenolic compounds from different apple varieties using comprehensive two-dimensional liquid chromatography. *J. Chromatogr. A*, 1313, 275-283.

[12] Miura, T., Chiba, M., Kasai, K., Nozaka, H., Nakamura, T., Shoji, T., Kanda, T., Ohtake, Y. & Sato, T. (2008). Apple procyanidins induce tumor cell apoptosis through mitochondrial pathway activation of caspase-3. *Carcinogenesis*, 29, 585-593.

[13] Akiyama, H., Sato, Y., Watanabe, T., Nagaoka, M. H., Yoshioka, Y., Shoji, T., Kanda, T., Yamada, K., Totsuka, M., Teshima, R., Sawada, J.,

Goda, Y. & Maitani, T. (2005). Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. *FEBS Lett.*, 579, 4485-4491.

[14] Osada, K., Funayama, M. & Fuchi, S. (2006). Effects of dietary procyanidins and tea polyphenols on adipose tissue mass and fatty acid metabolism in rats on a high fat diet. *J. Oleo Sci.*, 55, 79-89.

[15] Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T. & Ohtake, Y. (2007). Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *J. Agric. Food Chem.*, 55, 4604-4609.

[16] Lakowski, B. & Hekimi, S. (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*, 95, 13091-13096.

[17] Sunagawa, T., Shimizu, T., Kanda, T., Tagashira, M., Sami, M. & Shirasawa, T. (2011). Procyanidins from apples (*Malus pumila* Mill.) extend the lifespan of *Caenorhabditis elegans*. *Planta Med.*, 77, 122-127.

[18] Wilson, M. A., Shukitt-Hale, B., Kalt, W., Ingram, D. K., Joseph, J. A. & Wolkow, C. A. (2006). Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging Cell*, 5, 59-68.

[19] Wood, J. G., Rogina, B., Lavu, S., Howitz, K., Helfand, S. L., Tatar, M. & Sinclair, D. (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*, 430, 686-689.

[20] Valenzano, D. R., Terzibasi, E., Genade, T., Cattaneo, A., Domenici, L. & Cellerino, A. (2006). Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.*, 16, 296-300.

[21] Tissenbaum, H. A. & Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. Nature, 410, 227-230.

[22] Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.*, 82, 47-95.

[23] Cooke, M. S., Evans, M. D., Dizdaroglu, M. & Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.*, 17, 1195-1214.

[24] Finkel, T. & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, 408, 239-247.

[25] Lucas, D. T. & Szweda, L. I. (1998). Cardiac reperfusion injury: aging, lipid peroxidation, and mitochondrial dysfunction. *Proc. Natl. Acad. Sci. USA*, 95, 510-514.

[26] Dhalla, N. S., Temsah, R. M. & Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *J. Hypertens.*, 18, 655-673.

[27] Lakatta, E. G. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation*, 107, 490-497.

[28] Peskin, A. V. & Winterbourn, C. C. (2000). A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1). *Clin. Chim. Acta*, 293, 157-166.

[29] Ishimoto, H., Tai, A., Yoshimura, M., Amakura, Y., Yoshida, T., Hatano, T. & Ito, H. (2012). Antioxidative properties of functional polyphenols and their metabolites assessed by an ORAC assay. *Biosci. Biotechnol. Biochem.*, 76, 395-399.

[30] Sunagawa, T., Watanabe, K., Ozawa, Y., Nakashima, S., Kanda, T., Tagashira, M., Sami, M., Kaneko, T., Tahara, S., Nakaya, H., Shirasawa, T. & Shimizu, T. (2012). Apple polyphenols regulate mitochondrial superoxide generation and extend survival in a mouse model of dilated cardiomyopathy. *Int. J. Life Sci. Med. Res.*, 2, 46-51.

[31] Nojiri, H., Shimizu, T., Funakoshi, M., Yamaguchi, O., Zhou, H., Kawakami, S., Ohta, Y., Sami, M., Tachibana, T., Ishikawa, H., Kurosawa, H., Kahn, R. C., Otsu, K. & Shirasawa, T. (2006). Oxidative stress causes heart failure with impaired mitochondrial respiration. *J. Biol. Chem.*, 281, 33789-33801.

[32] Kahle, K., Kraus, M. & Richling, E. (2005). Polyphenol profiles of apple juices. *Mol. Nutr. Food Res.*, 49, 797-806.

[33] Kawakami, S., Matsuda, A., Sunagawa, T., Noda, Y., Kaneko, T., Tahara, S., Hiraumi, Y., Adachi, S., Matsui, H., Ando, K., Fujita, T., Maruyama, N., Shirasawa, T. & Shimizu, T. (2009). Antioxidant, EUK-8, prevents murine dilated cardiomyopathy. *Circ. J.*, 73, 2125-2134.

[34] Glasser, S. P., Clark, P. I. & Applebaum, H. J. (1979). Occurrence of frequent complex arrhythmias detected by ambulatory monitoring: findings in an apparently healthy asymptomatic elderly population. *Chest*, 75, 565-568.

[35] Aronow, W. S., Epstein, S., K.S., S. & Koenigsberg, M. (1987). Prevalence of arrhythmias detected by ambulatory electrocardiographic monitoring and of abnormal left ventricular ejection fraction in persons older than 62 years in a long-term health care facility. *Am. J. Cardiol.*, 59, 368-369.

[36] Tresch, D. D. & Thakur, R. K. (1998). Ventricular arrhythmias in the elderly. *Emerg. Med. Clin. North Am.*, 16, 627-648.

[37] Lakatta, E. G. (2003). Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises. Part III: Cellular and molecular clues to heart and arterial aging. *Circulation*, 107, 490-497.

[38] van der Loo, B., Bachschmid, M., Spitzer, V., Brey, L., Ullrich, V. & Luscher, T. F. (2003). Decreased plasma and tissue levels of vitamin C in a rat model of aging: Implications for antioxidative defense. *Biochem. Biophys. Res. Commun.*, 303, 483-487.

[39] Sunagawa, T., Shimizu, T., Matsumoto, A., Tagashira, M., Kanda, T., Shirasawa, T. & Nakaya, H. (2014). Cardiac electrophysiological alterations in heart/muscle-specific manganese-superoxide dismutase-deficient mice: Prevention by a dietary antioxidant polyphenol. *BioMed Res. Int.*, 2014, 704291.

[40] Brouillette, J., Clark, R. B., Giles, W. R. & Fiset, C. (2004). Functional properties of K^+ currents in adult mouse ventricular myocytes. *J. Physiol.*, 559, 777-798.

[41] Brunet, S., Aimond, F., Li, H., Guo, W., Eldstrom, J., Fedida, D., Yamada, K. A. & Nerbonne, J. M. (2004). Heterogeneous expression of repolarizing, voltage-gated K^+ currents in adult mouse ventricles. *J. Physiol.*, 559, 103-120.

[42] Matsuura, H. & Shattock, M. J. (1991). Effects of oxidant stress on steady-state background currents in isolated ventricular myocytes. *Am. J. Physiol.*, 261, H1358-H1365.

[43] Nakaya, H., Takeda, Y., Tohse, N. & Kanno, M. (1992). Mechanism of the membrane depolarization induced by oxidative stress in guinea-pig ventricular cells. *J. Mol. Cell Cardiol.*, 24, 523-534.

[44] Bhatnagar, A. (1995). Electrophysiological effects of 4-hydroxynonenal, an aldehydic product of lipid peroxidation, on isolated rat ventricular myocytes. *Circ. Res.*, 76, 293-304.

[45] Selkoe, D. J. (2007). Developing preventive therapies for chronic diseases: lessons learned from Alzheimer's disease. *Nutr. Rev., 65*, S239-S243.

[46] Murakami, K., Irie, K., Morimoto, A., Ohigashi, H., Shindo, M., Nagao, M., Shimizu, T. & Shirasawa, T. (2003). Neurotoxicity and physicochemical properties of Abeta mutant peptides from cerebral amyloid angiopathy: implication for the pathogenesis of cerebral amyloid angiopathy and Alzheimer's disease. *J. Biol. Chem.*, 278, 46179-46187.

[47] Toda, T., Sunagawa, T., Kanda, T., Tagashira, M., Shirasawa, T. & Shimizu, T. (2011). Apple procyanidins suppress amyloid β-protein aggregation. *Biochem. Res. Int.*, 2011, 784698.

[48] Hudson, S. A., Ecroyd, H., Kee, T. W. & Carver, J. A. (2009). The thioflavin T fluorescence assay for amyloid fibril detection can be biased by the presence of exogenous compounds. *FEBS J.*, 276, 5960-5972.

[49] Murakami, K., Masuda, Y., Shirasawa, T., Shimizu, T. & Irie, K. (2010). The turn formation at positions 22 and 23 in the 42-mer amyloid β peptide: The emerging role in the pathogenesis of Alzheimer's disease. *Geriatr Gerontol Int*, 10, S169-S179.

[50] Behl, C., Davis, J. B., Lesley, R. & Schubert, D. (1994). Hydrogen peroxide mediates amyloid β protein toxicity. *Cell*, 77, 817-827.

[51] Pasinetti, G. M., Ksiezak-Reding, H., Santa-Maria, I., Wang, J. & Ho, L. (2010). Development of a grape seed polyphenolic extract with anti-oligomeric activity as a novel treatment in progressive supranuclear palsy and other tauopathies. *J. Neurochem.*, 114, 1557-1568.

[52] MacDonald, M. E., Ambrose, C. M., Duyao, M. P., Myers, R. H., Lin, C., Srinidhi, L., Barnes, G., Taylor, S. A., James, M. & Groot, N., et al. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, 72, 971-983.

[53] Morley, J. F., Brignull, H. R., Weyers, J. J. & Morimoto, R. I. (2002). The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*, 99, 10417-10422.

[54] Briggs, A. D. M., Mizdrak, A. & Scarborough, S. P. (2013). A statin a day keeps the doctor away: comparative proverb assessment modelling study. *BMJ*, 347, f7267.

[55] Caton, P. W., Pothecary, M. R., Lees, D. M., Khan, N. Q., Wood, E. G., Shoji, T., Kanda, T., Rull, G. & Corder, R. (2010). Regulation of vascular endothelial function by procyanidin-rich foods and beverages. *J. Agric. Food Chem.*, 58, 4008-4013.

[56] Nishizuka, T., Fujita, Y., Sato, Y., Nakano, A., Kakino, A. & Ohshima, S. (2011). Procyanidins are potent inhibitors of LOX-1: a new player in the French Paradox. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.*, 87, 104-113.

[57] Byun, E. B., Ishikawa, T., Suyama, A., Kono, M., Nakashima, S., Kanda, T., Miyamoto, T. & Matsui, T. (2012). A procyanidin trimer, C1, promotes NO production in rat aortic endothelial cells via both

hyperpolarization and PI3K/Akt pathways. *Eur. J. Pharmacol.*, 692, 52-60.

[58] Yoshioka, Y., Akiyama, H., Nakano, M., Shoji, T., Kanda, T., Ohtake, Y., Takita, T., Matsuda, R. & Maitani, T. (2008). Orally administered apple procyanidins protect against experimental inflammatory bowel disease in mice. *Int. Immunopharmacol.*, 8, 1802-1807.

[59] Yamashita, Y., Okabe, M., Natsume, M. & Ashida, H. (2013). Cinnamtannin A2, a tetrameric procyanidin, increases GLP-1 and insulin secretion in mice. *Biosci. Biotechnol. Biochem.*, 77, 888-891.

[60] Williamson, G. & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.*, 81, 243S-255S.

[61] Shoji, T., Masumoto, S., Moriichi, N., Akiyama, H., Kanda, T., Ohtake, Y. & Goda, Y. (2006). Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the porter method and high-performance liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem.*, 54, 884-892.

[62] Shinmura, K., Tamaki, K. & Bolli, R. (2008). Impact of 6-mo caloric restriction on myocardial ischemic tolerance: possible involvement of nitric oxide-dependent increase in nuclear Sirt1. *Am. J. Physiol. Heart Circ. Physiol.*, 295, H2348-H2355.

[63] Denis, M. C., Furtos, A., Dudonné, S., Montoudis, A., Garofalo, C., Desjardins, Y., Delvin, E. & Levy, E. (2013). Apple peel polyphenols and their beneficial actions on oxidative stress and inflammation. *PLoS One*, 8, e53725.

[64] Lu, Y. & Yeap Foo, L. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.*, 68, 81-85.

[65] Oszmianski, J., Wojdylo, A. & Kolniak, J. (2009). Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity, and turbidity of cloudy apple juice. *J. Agric. Food Chem.*, 57, 7078-7085.