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ORIGINAL PAPER

# Mitochondrial dysfunction in platelets and hippocampi of senescence-accelerated mice

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**Abstract** Senescence-accelerated mice (SAM) strains are useful models to understand the mechanisms of age-dependent degeneration. In this study, measurements of the mitochondrial membrane potential ($\Delta\psi_{\mathrm{m}}$) of platelets and the adenosine 5′-triphosphate (ATP) content of hippocampi and platelets were made, and platelet mitochondria were observed in SAMP8 (faster aging mice) and SAMR1 (aging resistant control mice) at 2, 6 and 9 months of age. In addition, an A$\beta$-induced (Amyloid beta-protein) damage model of platelets was established. After the addition of A$\beta$, the $\Delta\psi_m$ of platelets of SAMP8 at 1and 6 months of age were measured. We found that platelet $\Delta\psi_m$, and hippocampal and platelet ATP content of SAMP8 mice decreased at a relatively early age compared with SAMR1. The platelets of 6 month-old SAMP8 showed a tolerance to A$\beta$-induced damages. These results suggest that mitochondrial dysfunction might be one of the mechanisms leading to age-associated degeneration in SAMP mice at an early age and the platelets could serve as a biomarker for detection of mitochondrial function and age related disease.

**Keywords** Aging · Adenosine 5′-triphosphate · Mitochondria · Mitochondrial membrane potential · Senescence-accelerated mice

**Abbreviations** AD: (Alzheimer's disease) · ADP/O: (Adenosine diphosphate/oxygen) · ATP: (Adenosine 5′-[tetrahydrogen] triphosphate) · A$\beta$: (amyloid beta-protein). BDS: (base deactivated silica) · BP: (Band-pass filter) · Complex I: (NADH dehydrogenase) · Complex III: (cytochrome *c*-oxidoreductase) · Cu,Zn-SOD 1-3: (Copper zinc superoxide dismutase) · DMSO: (Dimethyl sulfoxide) · DNA: (deoxyribonucleic acid) · EDTA: (ethylenediaminetetraacetic acid) · FL: (Fluorescence channel) · HPLC: (High performance liquid chromatography · JC-1: (5,5′,6,6′-tetrachloro-1, 1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide) · Mn-SOD: (manganese superoxide dismutase) · NADH: (Nicotinamide adenine dinucleotide, reduced) · PRP: (platelet-rich plasma) · PMT: (PhotoMultiplier tube) · PBS: (Phosphate buffer solution) · Redox: (oxidation/reduction reaction) · SAM: (senescence-accelerated mice) · SAMP: (strains of accelerated senescence-prone mice) · SAMP8: (a substrain of SAMP) · SAMR: (strains of accelerated senescence-resistant mice) · SAMR1: (one of the three substrains of SAMR mice) · SPSS: (Statistical package for the social sciences) · TPP: (tetraphenyl-phosphonium) · 8-OHG: (8-hydroxyguanosine) · $\Delta\psi_{\mathrm{m}}$ (mitochondrial membrane potential)

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## Introduction

Aging is an inevitable biological process that leads to several characteristics, such as changes in the biochemical composition and function of tissue, increased mortality after maturation, progressive decline in physiological capacity, and reduced ability to respond adaptively to environmental stimuli with increased susceptibility and vulnerability to diseases (Troen, 2003). There are several theories of aging, such as error-catastrophe, protein modification, free radical (oxidative stress), and others (Satoh et al., 2004). Among

them, the mitochondrial theory of aging remains to be one of the most popular theories (Passos et al., 2006). The central postulate of moving time averages (MTA) states that attenuation of cellular bioenergetics is the leading cause of aging (Trubitsyn, 2006), and aging is associated with a marked decline in mitochondrial function, characterized by a decrease in oxidative phosphorylation and ATP (Adenosine 5′-[tetrahydrogen]triphosphate) synthesis, an increase in mtDNA (mitochondrial deoxyribonucleic acid) mutations, an increase in abnormal mitochondrial cristae structures and a marked rise in free radical production, all of which may predispose to age-related disorders (Pallotti et al., 1996; Cortopassi and Wong, 1999).

Many investigators have developed models for studying human aging (Kuro-o, 2001). Among them are senescence-accelerated mice (SAM), one of the murine models of accelerated aging. Established by Takeda et al. (review in 1997), SAM are frequently used to study the aging process and are comprised of nine inbred strains of accelerated senescence-prone mice (SAMP) and three accelerated senescence-resistant mice (SAMR). Compared with the SAMR strains, SAMP individuals show an irreversible advancement of senescence that is manifested by clinical signs and gross lesions such as alteration in general behaviour, degenerative arthritis, hair loss and increased skin coarseness (Alvarez-Garcia et al., 2006). In addition, SAMP8, one of the nine substrains of SAMP, exhibits a short life span, age-associated spontaneous deterioration in learning and memory (Miyamoto et al., 1992), and various pathological features of age-associated neurodegeneration (Kawamata et al., 1997). In contrast, one of the three substrains of SAMR, the SAMR1 strain, although closely related genetically to the SAMP8 strain, does not exhibit these early alterations of senescence (Nishikawa et al., 1998).

Park et al. (1996) reported that mitochondrial Cu,Zn-SOD 1-3 (Copper zinc superoxide dismutase) activities decreased in livers of SAMP1 mice from a very young age to old age, as compared to SAMR1 mice, and proposed mitochondrial dysfunction as a factor contributing to the senescence acceleration. After their work, several studies supported the notion of mitochondrial dysfunction as observed in SAMP mice. Kurokawa et al. (2001) also observed that the manganese superoxide dismutase (Mn-SOD) activity in the cerebral cortex of 10-week-old SAMP8 mice decreased by about 50% as compared to age-matched SAMR1 mice. However, there was no difference in the expression of this protein between the two strains, suggesting a post-translational modification might have reduced the enzymatic activity. Nakahara et al. (1998) studied oxidative phosphorylation in the livers of SAMP8 and SAMR1 mice. They found that the respiratory control ratio decreased during aging in SAMP8 mice, and it was estimated that by 18 months of age, there was insufficient ATP synthesis for normal cell metabolism. They also observed that the amount of the apoptotic regulating protein Bcl-x in the liver mitochondria was slightly decreased in SAMP8 mice. Milder effects of aging on mitochondrial functional parameters were observed in SAMR1 mice. Nishikawa et al. (1998) also observed a higher oxidation/reduction reaction (redox) state and higher activity of mitochondrial respiration with a lower respiration control ratio in the mitochondrial fractions from the brains of 2-month-old SAMP8 males as compared to SAMR1 mice. In the electron transport system, decreased activities of Complex I (NADH [Nicotinamide adenine dinucleotide, reduced] dehydrogenase) and Complex III (cytochrome *c*-oxidoreductase) were observed (Fujibayashi et al., 1998). Fujibayashi et al. (1998) observed a small but significantly greater amount of multiple mitochondrial DNA deletions in SAMP8 mice brains at 4 and 8 weeks of age.

Recently, blood platelets have been widely proposed as periphery biomarkers of mitochondrial function and aging-related diseases (Merlo Pich et al., 1996; Lenaz et al., 1998; Schapira, 1998). Since platelets possess mitochondria, they can be collected by minimally invasive procedures and therefore used for human research. The rationale of employing platelets as a biomarker for mitochondrial lesions (Holmsen, 1987) rests on the assumption that alterations occurring in senescence and age-related diseases may be present in all cells, and that platelet changes may signal generalized bioenergetic deficiencies (Aurelio et al., 2001).

In this work using SAMP8 and SAMR1 at 2, 6 and 9 months of age, the ATP content and $\Delta\psi_m$ of platelets are measured and compared with the ATP content of hippocampi. Also, an A$\beta$-induced damage model of platelets is established. After the addition of A$\beta$, the platelet mitochondrial membrane potentials ($\Delta\psi_m$) of 6-month old SAMP8 are measured against SAMP8 at 1 month old to test the tolerance of platelet mitochondria of aging SAMP8 mice for A$\beta$-induced damages. Through these experiments, we hope to explore the possible mechanisms underlying the senescence acceleration of SAMP strains.

## Materials and methods

### Solutions

A$\beta$ 1-42 (Amyloid beta-protein 1-42) (Chemicon, USA) was stored at $-20^{\circ}$C before use, and was dissolved in High Performance Liquid Chromatography (HPLC) grade water (about 6 mg/ml) then diluted to 1 mg/ml with PBS (Phosphate Buffer Solution) when in use. JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanineiodide) (Sigma, USA) was dissolved in Dimethyl Sulfoxide (DMSO) at 1mg/ml and stored at $-20^{\circ}$C before use.

### Mice

SAMP8 (a substrain of senescence accelerated mice) and SAMR1 (mice from the senescence resistant substrain of SAM) were provided by the Animal facility of Tianjin University of Traditional Chinese Medicine. They were housed under standard conditions, 25°C with a 12 h light/dark cycle, and allowed free access to water and a standard diet.

Eighteen SAMP8 and 18 SAMR1 at 2, 6 and 9 months of age were used for hippocampal and platelet ATP content measurement and platelet mitochondrial membrane potential ($\Delta\psi_m$) determination (six of each age group). Four SAMP8 at 1 month of age and six SAMP8 at 6 months of age were used for the establishment of A$\beta$-induced damage model of platelets.

### Sample preparation

Platelet-rich plasma (PRP) was prepared from blood obtained by puncture of the right ventricle from mice anesthetized with 10 g/L sodium pentobarbital. Blood samples were collected in monoject tubes containing EDTA (ethylenediaminetetraacetic acid) (5 mmol/L), and immediately centrifuged at 150$g$ for 20 min at 4°C. The supernatant was PRP. The platelet amount was adjusted to $110^8$/ml with platelet-free plasma (obtained by centrifugation of the blood sample at 4000$g$ for 20 min). All the PRP was stored at 4°C until use. Before platelet ATP content and $\Delta\psi_m$ measurement, platelets were prepared by centrifugation at 400$g$ for 15 min. For platelet ATP content measurement, purine extraction was prepared as described previously (Edenbrandt and Murphy, 1990).

For hippocampal ATP content measurement, the mice were exsanguinated and immediately decapitated for brain removal. The hippocampi were removed and quickly dissected on a cold plate at −20°C. Purine extraction was then performed as described previously (Manfredi et al., 2002).

### Measurement of hippocampal and platelet ATP content and platelet $\Delta\psi_m$

Hippcampal and platelet ATP content were measured by reversed-phase high-performance liquid chromatography (HPLC) (Waters Corporation, USA). Chromatographic conditions were as follows: the gradient elution was performed on a 4.6-mm-i.d. ×30-mm, 5-$\mu$m-particle-size hypersil BDS (base deactivated silica) C-18 HPLC column with buffer A at a rate of 0.8 ml/min. Buffer A (150 mM $NaH_2PO_4$) with pH adjusted to 6.45 by KOH (potassium hydroxide) was filtered through a Rainin 0.2-$\mu$m Nylon-66 filter (Rainin Instruments Co, Woburn, MA, USA) and degassed in a flask linked with a vacuum pipe. Twenty microliters of standard mixture were first subjected to chromatography to obtain the retention time (about 13 min) and then ultraviolet (UV) monitored at 254 nm for about 13min. Afterward, 20 $\mu$l of the sample was injected into the injection port of the HPLC. Peaks were identified by their retention times and by using cochromatography standards.

Platelet $\Delta\psi_m$ was determined by staining platelets with JC-1, which were then measured by flow cytometry (Coulter Epics Altra™, USA) as previously described (Perrotta et al., 2003). The PMT value of the detector in FL1 (Florescence Channel 1) was set at 525BP (Band Pass Filter); the FL2 PMT was set at 575BP. Data were analyzed with Expo32™ software to quantify the percentages of red and green fluorescence. The results were expressed as the ratio of the red fluorescence to the sum of the red and green fluorescence.

### Model preparation

The A$\beta$-induced damage model of platelets was prepared by adding A$\beta$ to platelet rich plasma at the final concentration of 2.5 $\mu$g/ml or 12.5 $\mu$g/ml and incubating at 4°C for 24 h. Subsequently, the platelet $\Delta\psi_m$ was determined as described above.

### Statistical analysis

Statistical analysis was done by SPSS 12.0 software (Statistical Package for the Social Sciences). All data were submitted to the Kolmogovor-Smirnov test for normal distribution and expressed as the mean ± SEM. For single variable comparisons, Student's $t$-test was used. For multiple variable comparisons, data were analyzed by one-way analysis of variance (ANOVA) followed by a Student-Newman-Keul's test.

## Results

### The change of platelet $\Delta\psi_m$ during aging

Platelet $\Delta\psi_m$ could be used as an indicator for mitochondrial integrity and bioenergetic function. Changes in the $\Delta\psi_m$ were determined with the lipophilic dye JC-1. Binding to mitochondria was detected by a shift in fluorescence from green, which is characteristic of its monomeric form, to red, which reflected florescent aggregation in mitochondria. Therefore, platelets stained with JC-1 exhibited a heterogeneous distribution of polarized (red fluorescing JC-1 aggregates) and depolarized (green fluorescing monomers) mitochondria (Figs. 1–4).

The change of the proportion of these mitochondrial subpopulations reflected mitochondrial functional states. Specifically, fluorescence in PMT3 (red direction) decreased with a corresponding increase in PMT2 (green direction)

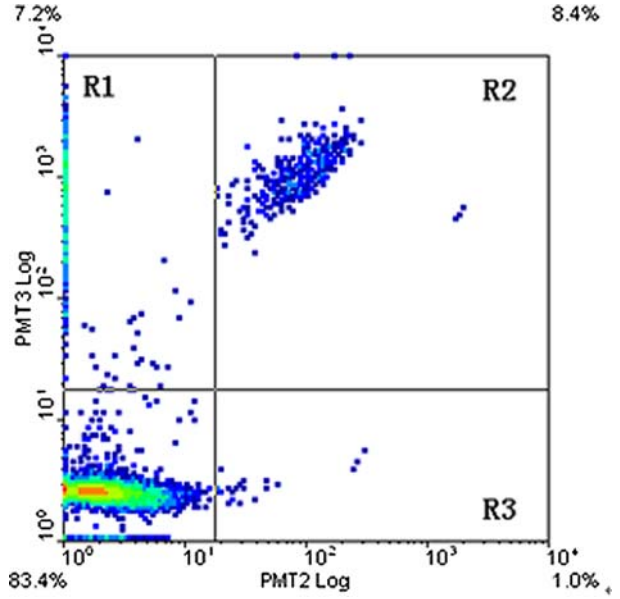


**Fig. 1** Representative flow cytometry dot plots showing JC-1 staining of platelets of SAMP8 at 1 month old. Approximately 7.2 percent of the platelet events express red J-aggregates (PMT3, as shown in the R1 gate) and 1 percent contains green monomer fluorescence (PMT2, as shown in the R3 gate)

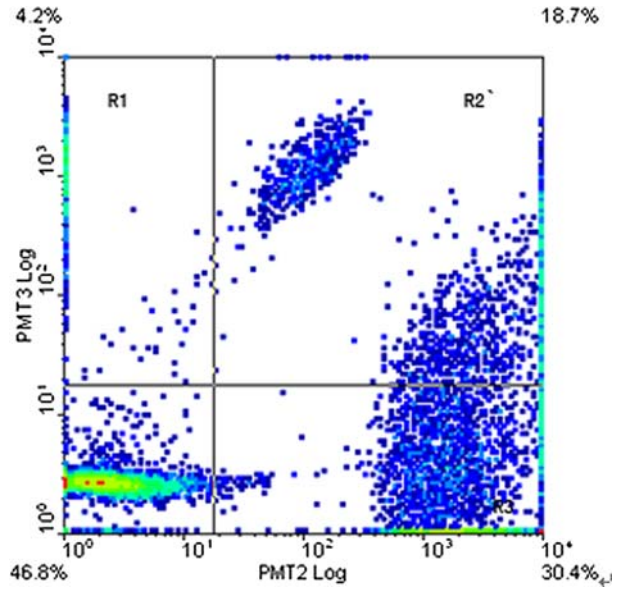


**Fig. 2** Representative flow cytometry dot plots showing JC-1 staining of platelets of SAMP8 at 1 month old when A$\beta$ was added at 12.5 $\mu$g/ml. Approximately 4.2 percent of the platelet events express red J-aggregates (PMT3, as shown in the R1 gate) and 30.4 percent contains green monomer fluorescence (PMT2, as shown in the R3 gate)

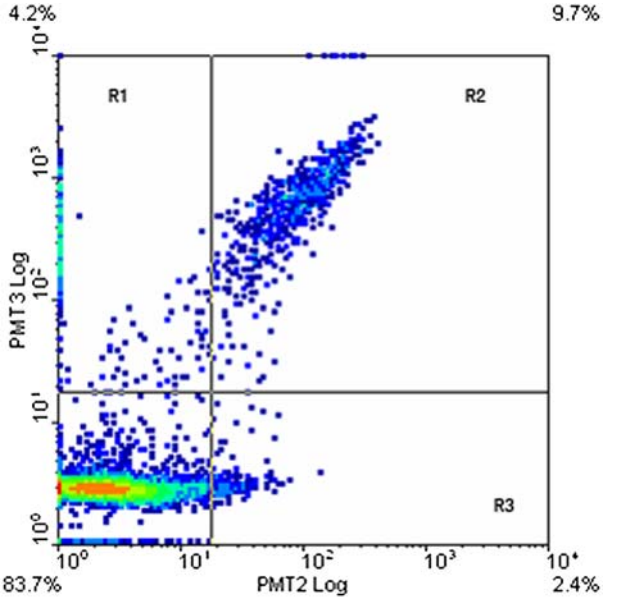


**Fig. 3** Representative flow cytometry dot plots showing JC-1 staining of platelets of SAMP8 at 6 month old. Approximately 4.2 percent of the platelet events express red J-aggregates (PMT3, as shown in the R1 gate) and 2.4 percent contains green monomer fluorescence (PMT2, as shown in the R3 gate)

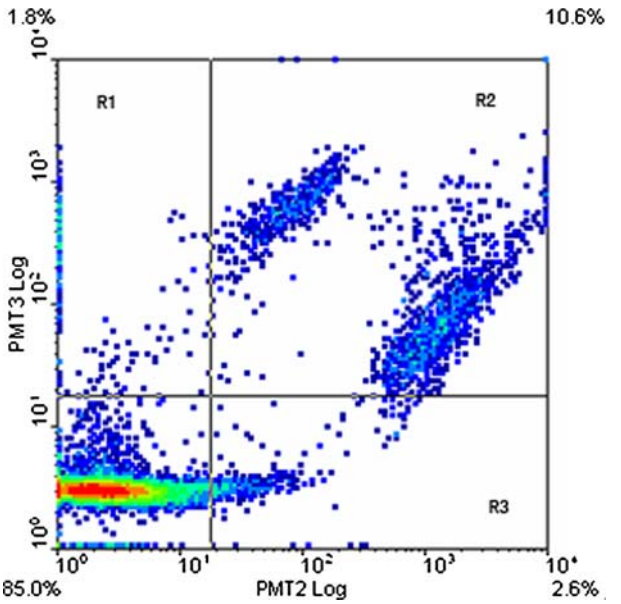


**Fig. 4** Representative flow cytometry dot plots showing JC-1 staining of platelets of SAMP8 at 6 month old when A$\beta$ was added at 12.5 $\mu$g/ml. Approximately 1.8 percent of the platelet events express red J-aggregates (PMT3, as shown in the R1 gate) and 2.6 percent contains green monomer fluorescence (PMT2, as shown in the R3 gate)

fluorescent intensity suggesting a depolarization decrease of platelet mitochondria (Figs. 1–4).

In this study, $\Delta\psi_m$ was measured in platelet mitochondria of SAMP8 and SAMR1 at 2, 6, and 9 months of age (Table 1). The following Student's *t*-tests showed that platelet $\Delta\psi_m$ of SAMP8 at 2, 6 and 9 months old were lower than SAMR1 at 2, 6 and 9 months old (compared with SAMR1of the same age, $p < 0.05$). In SAMP8 mice, at 6 months of age, platelet $\Delta\psi_m$ increased (compared with SAMP8 at 2 and 9 months old, $p < 0.05$) but decreased dramatically at 9 months of age (compared with SAMP8 at 2 and 6 months old, $p < 0.05$) (Table 1). In contrast, platelet $\Delta\psi_m$ of SAMR1showed no

**Table 1** Comparison of platelet mitochondrial membrane potential of SAMP8 and R1 mice of different age groups

| | Red fluorescence/total summation of red and green fluorescence (%) | | |
|---|---|---|---|
| | 2 months | 6 months | 9 months |
| SAMP8 | $0.75 \pm 0.02^{a}$ | $0.87 \pm 0.04^{a,b}$ | $0.59 \pm 0.01^{a,c}$ |
| SAMR1 | $0.90 \pm 0.03$ | $0.93 \pm 0.02$ | $0.70 \pm 0.02^{d}$ |

[a]Compared with SAMR1 of the same age, $p < 0.05$; [b]compared with SAMP8 at 2, 9 months old, $p < 0.05$; [c]compared with SAMP8 at 2, 6 months old, $p < 0.05$; [d]compared with SAMR1 at 2, 6 months old, $p < 0.05$.

**Table 2** Comparison of platelet mitochondrial membrane potential of SAMP8 at 1, 6 months old after the addition of A$\beta$

| | Red fluorescence/total summation of red and green fluorescence (%) | | |
|---|---|---|---|
| SAMP8 | Control | 2.5 $\mu$g/ml A$\beta$ | 12.5 $\mu$g/ml A$\beta$ |
| 6 months | $0.82 \pm 0.06$ | $0.82 \pm 0.04$ | $0.59 \pm 0.18$ |
| 1 months | $0.67 \pm 0.10$ | $0.53 \pm 0.05$ | $0.34 \pm 0.07^{a}$ |

[a]compared with control group, $p < 0.05$.

significant differences between 2 and 6 months, but decreased at 9 months (compared with SAMR1 at 2 and 6 months old, $p < 0.05$) (Table 1).

After the addition of A$\beta$ in the platelet preparations, no significant differences of platelet $\Delta\psi_m$ of SAMP8 at 6 months were found in the control group, 2.5 $\mu$g/ml A$\beta$ group and 12.5 $\mu$g/ml A$\beta$ group ($p > 0.05$) (Table 2; Figs. 1 and 2.). On the other hand, there was a significant difference in platelet $\Delta\psi_m$ of SAMP8 at 1 month between the control group and 12.5 $\mu$g/ml A$\beta$ group (Figs. 3 and 4), though no significant difference between the control group and 2.5 $\mu$g/ml A$\beta$ group was found ($p > 0.05$) (Table 2).

ATP content of hippocampi during aging

ATP is a direct indicator of the energy metabolism of tissues. Our experiments indicated that no significant differences in hippocampal ATP content were observed between SAMP8 and SAMR1 at 2 months old ($p > 0.05$). However, the hippocampal ATP content of SAMP8 at 6 and 9 months old was lower than SAMR1 of the same ages ($p < 0.05$) (Table 3). In SAMP8 mice, the hippocampal ATP content showed no significant differences between 2 and 6 months ($p > 0.05$), but decreased dramatically at 9 months (compared with SAMR1 at 2 and 6 months old, $p < 0.05$). Similarly, the hippocampal ATP content of SAMR1 also showed no significant differences between 2 and 6 months ($p > 0.05$), but decreased at 9 months (compared with SAMR1 at 2 and 6 months old, $p < 0.05$) (Table 3).

**Table 3** Comparison of hippocampal ATP content of SAMP8 and R1 mice of different age groups

| | Hippocampal ATP content (mg/ml) | | |
|---|---|---|---|
| | 2 months | 6 months | 9 months |
| SAMP8 | $2.40 \pm 0.25$ | $2.42 \pm 0.15^{1}$ | $0.95 \pm 0.08^{a,b}$ |
| SAMR1 | $2.68 \pm 0.14$ | $2.81 \pm 0.12$ | $2.43 \pm 0.02^{c}$ |

[a]compared with SAMR1 of the same age, $p < 0.05$;[b]compared with SAMP8 at 2, 6 months old, $p < 0.05$;[c]compared with SAMR1 at 2, 6 months old, $p < 0.05$.

**Table 4** Comparison of platelet ATP content of SAMP8 and R1 mice of different age groups

| | Platelet ATP content (mg/ml) | | |
|---|---|---|---|
| | 2 months | 6 months | 9 months |
| SAMP8 | $12.90 \pm 3.78$ | $14.82 \pm 4.90$ | $5.85 \pm 0.70^{a,b}$ |
| SAMR1 | $14.27 \pm 2.98$ | $15.64 \pm 2.38$ | $13.79 \pm 0.50$ |

[a]compared with SAMR1 at 9 months old, $p < 0.05$;[b]:compared with SAMP8 at 2, 6 months old, $p < 0.05$; [c]:compared with SAMR1 at 2, 6 months old, $p < 0.05$.

ATP content of platelets during aging

In platelets, no significant differences in ATP content between SAMP8 and SAMR1 were observed at 2 and 6 months old ($p > 0.05$) (Table 4). However, the platelet ATP content of SAMP8 at 9 months old was lower than SAMR1 of the same age ($p < 0.05$). In SAMP8 mice, the platelet ATP content showed no significant change at 2 and 6 months ($p > 0.05$), but again decreased dramatically at 9 months (compared with SAMR1 at 2 and 6 months old, $p < 0.05$). Likewise, the platelet ATP content of SAMR1 also showed no significant change at 2 and 6 months ($p > 0.05$), and decreased at 9 months old (compared with SAMR1 at 2 and 6 months old, $p < 0.05$).

## Discussion

The mitochondrion sits at the centre of the web of bioenergetic interaction that controls much of cell function. Three fundamental functions, ATP generation, $Ca^{2+}$ uptake and storage, and the generation and detoxification of reactive oxygen species, are driven by the mitochondrial membrane potential, $\Delta\psi_m$ (Nicholls 2004).

Recently, JC-1 and cytofluorimetric technique has been wildly used for the measurement of $\Delta\psi_m$ (Perrotta et al. 2003; Bodo et al., 2006; Wang et al., 2006; Sukhodub et al., 2007). This method is relatively easy and precise, as JC-1 is more advantageous over rhodamines and other

carbocyanines, being capable of entering selectively into mitochondria. It can reverse its color from green to orange as membrane potentials increase (over values of approximately 80–100 mV) while the TPP+ electrode technique, though more accurate, requires discrete amounts of biological samples. In addition, the uptake of this lipophilic cation by intact mammalian cells is a slow process.

Our experiment showed that overall platelet $\Delta\psi_m$ of SAMP8 was lower than SAMR1 at 2, 6 and 9 months old. Specifically in SAMP8 mice, platelet $\Delta\psi_m$ was higher at 6 months than at 2 and 9 months old and the platelet $\Delta\psi_m$ at 9 months old was lowest of all. In contrast, platelet $\Delta\psi_m$ of SAMR1 showed no significant difference at 2 and 6 months, and then decreased at 9 months. These results suggest to us that mitochondrial dysfunction may exist in platelets of SAMP8 at an early age (2 months of age) and could be one of the mechanisms of accelerated senescence. However, in SAMP8 mice at 6 months of age, platelet $\Delta\psi_m$ increased while the platelet ATP content of SAMP8 at 6 months old did not show significant increase and finally decreased at 9 months old. These findings were consistent with the results of A$\beta$-induced damage model. The addition of A$\beta$ in platelets of SAMP8 and SAMR1 mice initially produced a significant difference in platelet $\Delta\psi_m$ in SAMP8 of 1 month between the control group and 12.5 $\mu$g/ml A$\beta$ group. In contrast, we found no significant differences in platelet $\Delta\psi_m$ of SAMP8 at 6 months between the control group and 12.5 $\mu$g/ml A$\beta$ group. It suggested to us that the tolerance of platelet mitochondria of 6 month old SAMP8 mice to A$\beta$-induced damage might be as a result of chronic mild stimulation, similar to amyloid $\beta$ precursor protein induced changes in platelets of patients with Alzheimer's Disease (Ashley and Rudolph, 1998; Bush and Tanzi, 1998; Blasko et al., 1999; Colciaghi et al., 2004). This finding would also support the hypothesis of a compensatory mechanism previously proposed by Reddy et al. and Manczak et al. in which mitochondrial gene up-regulation was found in the brains of mutant amyloid precursor protein (APP) transgenic mice at 2, 5 and 18 months of age (Reddy et al., 2004) and C57BL6 mice in the late stages of life. (Manczak et al., 2005). Some mitochondrial genes that up-regulated in non-transgenic mice in response to age-related A$\beta$ aggregation might effectively prevent plaque formation related to AD(Reddy et al., 2004). But in transgenic mice, this compensatory change has not been longlasting and will finally deteriorate (Reddy et al., 2004). Similarly, mitochondrial genes up-regulated in specimens from 12- and 18-month old C57BL6 mice allowed for more production of proteins involved in the electron transport chain to counter-balance the abnormal electron transport chain of the mitochondria, while the down-regulation of mitochondrial genes in later stages of aging (24 month old) indicated that the compensation maintained by the up-regulated genes could not be sustained (Manczak et al., 2005).

Although it has been reported that SAMP8 mice had age-related increases in the level of hippocampal A$\beta$ peptide (Morley et al., 2000; Petursdottir et al., 2006), the literature on A$\beta$-induced peripheral damages and SAMP mice is limited. In SAMR1, the decrease of $\Delta\psi_m$ appeared relatively late (at about 9 months old) and probably was the result of normal aging (Nicholls, 2004).

It had been reported that the number of mitochondria with low-membrane potential increased and the ratio of mitochondria with a high-membrane potential to the total number of mitochondria decreased in SAMP11 cells after the seventh day in culture (Harley and Goldstein, 1980; Goldstein, 1990; Cristofalo and Pignolo, 1993). An ultrastructural study showed that the mitochondria morphology altered concomitantly with the increase in the proportion of mitochondria with low-membrane potential (Harley and Goldstein, 1980; Goldstein, 1990; Cristofalo and Pignolo, 1993). Rottenberg and Wu (1997) and Wong et al. (2006) also reported that spleen lymphocytes from old mice have lower respiration rates and lower $\Delta\psi_m$ than lymphocytes from young mice because of an increase in oxidative stress. It had also been shown that T cells from old mice exhibit lower $\Delta\psi_m$ in a study with rhodamine 123 fluorescence (Rottenberg and Wu, 1997). In addition, in the liver cells from old rats, $\Delta\psi_m$ decreased while mitochondrial heterogeneity and oxidants increased (Rottenberg and Wu, 1997). Results showed that aging also decreased $\Delta\psi_m$ in hepatocytes (Hagen et al., 1997; Sastre et al., 1999; Sastre et al., 2000), human skin fibroblasts (Martinez et al., 1986; Martinez et al., 1987; Martinez et al., 1991), epithelial cells (Maftah et al., 1994), and neuronal and brain cells (Sugrue et al., 1999; Sugrue et al., 2001). These findings may further confirm the assumption that alterations occurring in senescence and age-related diseases may be present in all cells. Furthermore, in human tissues, Sastre et al. (2000) reported that mitochondrial size increased whereas mitochondrial membrane potential decreased with age in brain and liver.

The underlying basis for the decline in the $\Delta\psi_m$ with aging is unknown. Studies with liver mitochondria showed that aging increased proton leakage, inhibited respiration, and caused accumulation of mitochondrial oxidative damage (Kokoszka et al., 2001). Aged mitochondria showed a tissue specific reduction in the activity of electron-transport complexes (Kwong and Sohal, 2000). Mitochondria from old animals produced significantly more oxidants than those from younger animals (Hagen et al., 1997), which might diminish mitochondrial functional potential by damaging mitochondrial proteins. For example, ketoglutaric dehydrogenase complexes and aconitase (Yan et al., 1997) were targets of the reactive species, and their reduction would show diminished NADH (Nicotinamide adenine dinucleotide, reduced) production (Tretter and Adam-Vizi, 2000).

Reductions in $\Delta\psi_m$ were associated with changes in the conformation of membrane pore proteins. The mitochondrial permeability transition pore was more susceptible to activation in lymphocytes from old mice than those of young mice (Rottenberg and Wu, 1997). Opening of the permeability transition allowed release of cytochrome *c* and promoted apoptotic cascades. Calcium uptake was coupled to $\Delta\psi_m$, and calcium uptake by fibroblasts declined with the age of patients with Alzheimer's disease (Peterson et al., 1985a). Calcium uptake into mitochondria within isolated nerve endings declined with aging (Peterson et al., 1985b). The decline in $\Delta\psi_m$ might underlie the age-related decline in ATP production and oxygen consumption (Harper et al., 1998). Thus, differences in resting $\Delta\psi_m$ signal would alter multiple cell function.

Mitochondrial function and dysfunction in the central nervous system could be reflected in ATP production. Neuron energy production is derived mainly from oxidative phosphorylation of mitochondria. Our experiments demonstrated no significant differences of hippocampal ATP content between SAMP8 and SAMR1 at 2 months old. However, the hippocampal ATP content of SAMP8 at 6 and 9 months old became significantly lower than SAMR1 of the same ages. In SAMP8 or SAMR1 mice, the hippocampal ATP content showed no significant differences in either group at 2 and 6 months, but significantly decreased by 9 months. Similarly, it was reported that there were no significant differences of ATP content between SAMP8 and SAMR1 mice at 3 month old (Shimano, 1998). In addition, in the brain tissue of SAMP8, a marked transient enhancement of anaerobic glycolytic capacity occurred in 2-month-olds followed by a subsequent decrease in mitochondrial function, as a result of which glucose metabolism appears to be enhanced in both the 2- and 10-month-old SAMP8 when compared with SAMR1 mice (Omata et al., 2001). In addition, the transient overproduction of the glucose transporter protein in the cerebral cortex resulted in increased glucose metabolism in 4- to 8-week-old SAMP8 (Ozaki et al., 1996). The brain mitochondria of 2-month old SAMP8 mice demonstrated a higher redox state and higher activity of mitochondrial respiration with lower respiration control ratio than the mitochondria of SAMR1 brains of same age. This hyperactive state might relate to electron leakage in mitochondrial respiration during the growth phase, which might be a compensatory mechanism for underlying genetic, epigenetic or structural disorders, including membrane permeability changes (Nakahara et al., 1998; Nishikawa et al., 1998). Further, immunofluorescence analysis of the DNA damage marker (8-OHG or 8-hydroxyguanosine) and cytochrome *c* in the brain sections from 12- and 18-month-old C57BL6 mice, compared to 2-month-old mice revealed an increased immunoreactivity of 8-OHG and cytochrome c, suggesting that age-related mitochondrial oxidative damage and apoptosis were associated with mitochondrial dysfunction (Manczak et al., 2005). These findings may partly explain our results that no differences of hippocampal ATP content were observed between 2-month old SAMP8 and SAMR1 mice, but the final compromise of mitochondrial function with aging might lead to the decrease of ATP production.

Our experiments also found no significant differences of platelet ATP content between SAMP8 and SAMR1 at 2 and 6 months old, but both groups' platelet ATP content decreased at 9 months. On the other hand, the platelet ATP content of SAMP8 at 9 months old was lower compared with SAMR1 at this age. Unlike neurons, platelet energy production is derived from both glycolysis and oxidative phosphorylation of mitochondria (Holmsen, 1987), and platelet glycolysis would enhance correspondingly once the oxidative phosphorylation of mitochondria had been weakened. Therefore, compared with the change of $\Delta\psi_m$, the lowering of platelet ATP content of SAMP8 appeared later (at 9 months of age), probably due to an enhancement of platelet glycolysis, while the decrease of platelet ATP content at 9 months of age might be a result of the lowering of both mitochondrial function and gycolysis. In SAMR1, the decrease of both $\Delta\psi_m$ and ATP content of platelets was almost synchronous (both decreased at about 9 months old), probably as a result of normal aging (Nicholls, 2004).

Similarly, in other tissues, it was also reported that the ATP content of hearts of SAMP8 mice at 5 or 10 months of age was lower than SAMR1 at the same age (Rodriguez, 2007). The underlying basis of the decline of ATP content with aging remains unclear. Except for the effects of the decrease of $\Delta\psi_m$, there are probably other mechanisms involved. As mentioned above, Nakahara et al. (1998) reported that the respiratory control ratio decreased in liver mitochondria of SAMP8 during aging. It was estimated that at 18 months of age this respiratory control value might be insufficient to provide ATP synthesis necessary for normal cell metabolism. In addition, the ADP/O (Adenosine diphosphate/Oxygen), an index of efficiency of ATP synthesis, was depressed at 18 months of age. Dinitrophenol-dependent uncoupled respiration in liver mitochondria of SAMP8 mice markedly decreased with aging, suggesting a dysfunctional energy transfer mechanism in mitochondria of aged SAMP8 mice (Nakahara et al., 1998). Active uptake of calcium in liver mitochondria was dysfunctional in aged SAMP8 mice, and uncoupling of respiration was induced more easily in the aged mitochondria. Milder effects on these functional parameters were observed in SAMR1 mice. A similar dysfunction was also observed in heart mitochondria of SAMP8 mice at 12 months of age (Nakahara et al., 1998). In the electron transport system, decreased activities for Complex I and Complex III were also observed (Fujibayashi et al., 1998).

Therefore, although the exact aging mechanisms are still unclear for the senescence acceleration in SAMP strains, one possible mechanism that causes this degeneration in SAMP mice at an early age is indicated by mitochondrial dysfunction. In addition, from our experiments, platelet $\Delta\psi_m$ appeared more vulnerable than both hippocampal and platelet ATP content with aging and might have the potential to be employed clinically as an indicator of age-related diseases such as Alzheimer's disease.

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