Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer’s disease

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Although there are no proven ways to delay onset or slow progression of Alzheimer's disease (AD), studies suggest that diet can affect risk. Pomegranates contain very high levels of antioxidant polyphenolic substances as compared to other fruits and vegetables. Polyphenols have been shown to be neuroprotective in different model systems. We asked whether dietary supplementation with pomegranate juice (PJ) would influence behavior and AD-like pathology in a transgenic mouse model. Transgenic mice (APPsw/Tg2576) received either PJ or sugar water control from 6 to 12.5 months of age. PJ-treated mice learned water maze tasks more quickly and swim faster than controls. Mice treated with PJ had significantly less (\sim 50%) accumulation of soluble \beta amyloid and amyloid deposition in the hippocampus as compared to control mice. These results suggest that further studies to validate and determine the mechanism of these effects, as well as whether substances in PJ may be useful in AD, should be considered.

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Introduction

Alzheimer’s disease (AD) is the most common cause of dementia and affects more than 10% of individuals over the age of 65. Although there are currently no proven ways to delay the onset or slow the progression of AD, epidemiological and experimental evidence suggests that diet can affect the risk for AD and alter amyloid-\beta (A\beta) levels. For example, a high cholesterol diet has been shown to increase levels of A\beta and apoE (key constituents of the plaques deposited in the brains of AD patients) in the brains of rabbits (Wu et al., 2003) and APP transgenic mice (Refolo et al., 2000). It has been hypothesized that diets high in carbohydrates may alter metabolism of cellular membrane proteins (e.g., APP) and trigger excessive cell signaling cascades, leading to neuronal damage (Henderson, 2004). Other research suggests that dietary intake of aluminum may increase the risk of developing AD (Newman, 1992; Roberts et al., 1998; Rogers and Simon, 1999) and that diets deficient in magnesium can produce cognitive deficits in mice (Bardgett et al., 2005). Importantly, mounting evidence suggests that diet can also decrease the risk for developing AD (Mattson, 2000; Pope et al., 2003). Caloric restriction appears to be neuroprotective in mouse models of AD (Love, 2005; Wang et al., 2005) by decreasing the accumulation of A\beta deposits (Patel et al., 2005). Another recent study suggests that increased dietary intake of niacin may slow the progression of cognitive decline in AD (Morris et al., 2004).

Foods containing high levels of antioxidants may also slow the progression of AD, possibly by preventing or neutralizing the damaging effects of free radicals (Kostrzewa and Segura-Aguilar, 2003; Polidori, 2003). The essential fatty acids contained in fish oil (e.g., docosahexaenoic acid/DHA) may be neuroprotective in humans (Grant, 2000, 2003; Horrocks and Yeo, 1999; Peers, 1990). Recent studies have also shown beneficial effects of DHA on learning in a rat model of AD (Hashimoto et al., 2002, 2005) and on both plaque deposition and dendritic pathology in aged APPsw transgenic mice (Calon et al., 2004; Lim et al., 2005). Chronic dietary administration of the antioxidant vitamin E has been shown to reduce A\beta deposits in APPsw mice (Sung et al.,...
and epidemiological evidence suggests that high intake of food-based vitamin E is associated with a lower incidence of AD in humans (Morris et al., 2005).

Phytochemicals are nonnutritive bioactive chemicals found in plants (especially pigments) that can have beneficial effects on health. Phytochemicals like polyphenols (including the phenolic acids and flavonoids) have been shown to have antioxidant properties and to suppress inflammatory and other pathways (Aggarwal and Shishodia, 2004; Joseph et al., 2005). Quercetin, a flavonoid polyphenol found in several fruits and vegetables, was recently shown to protect against oxidative stress in vitro (Heo and Lee, 2004), and curcumin, a polyphenol found in the curry spice turmeric, was shown to lower levels of oxidized proteins and plaque burden in APP<sub>sw</sub> mice (Lim et al., 2001). Green tea, another food high in polyphenols, may also be neuroprotective (Weinreb et al., 2004), and one of its flavonoid components, epigallocatechin-3-gallate, decreased Aβ levels in APP<sub>sw</sub> mice (Rezai-Zadeh et al., 2005). Dietary supplementation with blueberries, also rich in polyphenols, has been shown to improve Y-maze performance, but not plaque deposition, in APP+PS1 transgenic mice (Joseph et al., 2003).

Pomegranates contain very high levels of polyphenols as compared to other fruits and vegetables (Kelawala and Ananthanarayan, 2004; Wang et al., 2004; Xu et al., 2005). Dietary supplementation of pregnant mice with pomegranate juice was recently shown by our laboratory to protect against neurodegeneration in neonatal mice subjected to hypoxic–ischemic brain injury (Loren et al., 2005). Therefore, we asked whether dietary supplementation with pomegranate juice would influence AD-like pathology and behavior in a mouse model of AD.

Materials and methods

**Animals—Transgenic experiments**

Beginning at 6months of age, transgenic mice expressing a form of the amyloid precursor protein (APP) that causes early-onset familial AD (APP<sub>sw</sub>/Tg2576) (Hsiao et al., 1996) received in their drinking bottles pomegranate juice (PJ) from a single lot of PJ concentrate (PomWonderful; Los Angeles, CA) diluted 1:160 or 1:80 in filtered water. Since the PJ concentrate is 4 times more concentrated than regular strength PJ sold commercially, the dilutions of concentrate are approximately equivalent to dilutions of 1:40 or 1:20 of non-concentrated PJ. The mice drank an average of 5ml of fluid per day. This amount of PJ at these dilutions is roughly equivalent on a mg/kg basis to a human drinking one versus two 8-ounce glasses of PJ per day. The amount of polyphenols consumed per day was estimated to be ~0.3–0.6mg. Control APP<sub>sw</sub> mice received sugar water that mimicked the sugar content of the 1:40 PJ (85% sucrose, 7.5% D(+)-glucose, 7.5% D-fructose). Previous data from our laboratory have shown that polyphenolic substances in PJ are detectable in the plasma of mice pups whose mothers drank PJ but not in pups whose mothers drank sugar water (Loren et al., 2005). Because there were no statistically significant differences between the 1:40 and 1:20 PJ groups for any of the behavioral or neuropathological assessments, the two PJ groups were grouped together for purposes of data analysis. Additionally, there were no weight differences between any of the groups. Behavioral testing began at 11.5months of age, and the mice continued their treatment regimen throughout testing.

**Animals—Wildtype experiments**

Wildtype (non-transgenic) control littermates of the APP<sub>sw</sub> mice were fed PJ (1:40 dilution) or vehicle (sugar water control) beginning at 3–5months of age. There were no weight differences between the groups. Behavioral testing began after 3 weeks of treatment, and the mice continued their treatment regimen throughout testing.

**Pomegranate juice preparation**

Pomegranates (Wonderful variety) were picked by hand, washed, and stored in tanks. The fruit was crushed and squeezed. The juice was filtered, pasteurized, concentrated and stored at −18°C. The composition of PJ that was used in these experiments was assessed as follows. PJ was fractionated by low-pressure chromatography on Sephadex LH-20. Five different fractions were obtained by sequential elution with aqueous/organic solvents and then analyzed by high-performance liquid chromatography (HPLC), diode array ultraviolet detection (DAD), electrospray ionization-mass spectrometry (ESI-MS), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (J. Reed, University of Wisconsin, Madison, personal communication). PJ consisted of 84% water, 14% carbohydrates, 0.48% ash, 0.4% citric acid, 0.1% protein, 0.02% fat and 1% other, including polyphenols (phenolic acids and flavonoids). Phenolic acids included 115ppm ellagic acid and 5ppm gallic acid. Flavonoids included 1880ppm hydrolysable tannins (e.g., gallotannins, ellagitannins, punicalagin) and 369ppm anthocyanins and their glycosides (e.g., cyanidin, delphinidin, pelargonidin).

**Water maze**

The learning and memory abilities of the mice were tested in the Morris water maze, as described (Hartman et al., 2005). Briefly, this test of spatial navigation learning requires the mouse to find a hidden (submerged) platform in a pool of water using visual cues from around the room. As performance improves, escape latency and swim path length generally decrease. The water maze consisted of a metal pool (118 cm diameter) in a well-lit room filled to within 10 cm of the upper edge with water made opaque by the addition of white non-toxic tempera paint. The pool contained a round platform (22 cm diameter) that the mice could step on to escape the water. For each trial, a mouse was released nose against the wall into the pool at one of four release points and allowed to find the platform. All trials lasted a maximum of 60s, at which point the mouse was manually guided to the platform. An overhead camera recorded the animals’ swim paths, allowing for quantification of distance, latency, proximity to target and swimming speed by a computer (Polytrack; San Diego Instruments, San Diego, CA).

**CUED water maze**

The CUED (visible platform) task was used to assess sensorimotor and/or motivational deficits that could affect performance during the SPATIAL water maze task. For this task, the surface of the escape platform was visible (5 mm above the surface of the water), and a 10 cm tall pole capped by a red tennis ball was placed on top of the platform to make its location even more obvious. The walls of the room were kept bare, although the room geometry, experimenter and computer system were obvious
Spatial cues. The mice were given four consecutive trials per day, each time with the platform in the middle of a different pool quadrant. The mouse was released into the pool opposite the location of the platform for that trial. After each trial, the mouse was placed into a holding cage for 30s while the platform was moved to its next location. For the APP\textsubscript{sw} mice, CUED testing continued for 5 days, giving each mouse a total of 20 CUED trials. For the wildtype mice, CUED testing continued for 3 days, giving each mouse a total of 12 CUED trials. Mice that displayed behaviors inappropriate for SPATIAL water maze testing (including spinning, thigmotaxic navigation around the perimeter of the pool and inability to swim) were removed from the study (APP\textsubscript{sw}: 3 of 16 control mice and 4 of 32 PJ mice; wildtype: 2 of 14 control and 3 of 13 PJ mice). This left sample sizes of 13 controls/28 PJ APP\textsubscript{sw} mice and 12 vehicle/10 PJ wildtype mice for continued spatial testing.

**SPATIAL water maze**

Three days after the conclusion of CUED testing, SPATIAL (hidden platform) testing began. For this task, the surface of the escape platform was submerged 1 cm below the surface of the water, and several spatial cues were added to the walls of the room, which required the mice to find the platform based on its relationship to the cues rather than direct visualization. Four consecutive trials were administered per day for the study (APP\textsubscript{sw}: 3 of 16 control mice and 4 of 32 PJ mice; wildtype: 2 of 14 control and 3 of 13 PJ mice). This left sample sizes of 13 controls/28 PJ APP\textsubscript{sw} mice and 12 vehicle/10 PJ wildtype mice for continued spatial testing.

**Histology and tissue dissection**

At 12.5 months of age, the APP\textsubscript{sw} mice were perfused through the heart with PBS, and their brains were removed. The left hemisphere of each brain was immersed in 4% paraformaldehyde in 0.1M PBS at 4°C for 24h and then soaked in a 30% sucrose solution at 4°C for 24h, followed by freezing in powdered dry ice. The brain was then cut coronally in 50μm sections from the genu of the corpus callosum to the end of the hippocampal formation. A subset of the sections (every 6th) was stained with pan anti-β\textsubscript{Aβ} antibody (Biosource International; Camarillo, CA), and another subset of every 6th section was stained for fibrillar β\textsubscript{Aβ} (amyloid) using thioflavine-S, as described (Hartman et al., 2005). The hippocampus and dorsal cortex (defined as the cortical tissue overlying the hippocampus) of each animal were assessed for β\textsubscript{Aβ} and fibrillar β\textsubscript{Aβ} load (i.e., percent area covered by deposits) using an unbiased stereological method (area fraction fractionator; Stereo Investigator, Microbrightfield; VT) and a Nikon E800 microscope. To examine the effects of PJ on cerebral amyloid angiopathy (CAA; a common neuropathological condition in humans with AD and in the APP\textsubscript{sw} mouse (Fryer et al., 2005)), thioflavine-S-positive blood vessels were quantified in the dorsal cortex. To further assess whether PJ had a protective effect on neuronal processes near fibrillar β\textsubscript{Aβ} deposits, thioflavine-S stained brain sections were double-labeled with a silver stain as described. Numbers of dystrophic/swollen neurites (>2.5μm in diameter) were counted at the site of each thioflavine-S positive plaque to attain an average number of dystrophic neurites per plaque. The hippocampus and cortex were dissected from the right hemisphere of each brain and frozen at −80°C for later biochemical analyses.

**Aβ ELISAs**

Tissue was prepared for ELISA as described (DeMattos et al., 2002). Cortices and hippocampi were homogenized in carbonate buffer, spun down, and the supernatant was collected. The remaining pellet was re-homogenized in 5M guanidine buffer, spun down, and the supernatant was collected. Aβ\textsubscript{40} and Aβ\textsubscript{42} levels were assessed with Biosource International Aβ ELISA kits (Camarillo, CA).

**APP Western blots**

To assess APP/Aβ processing, cortices were processed as described (Wahrle et al., 2005). Briefly, the tissue was sonicated in 10μl/mg RIPA buffer with protease inhibitors and spun at 20,000×g for 25 min. The supernatant was collected, and protein levels were measured with a bicinchoninic acid (BCA) assay (Pierce; Rockford, IL). The samples were then run on 4–12% Bis–Tris gels with MES running buffer (Invitrogen; Carlsbad, CA). Following electrophoresis, proteins in the gels were transferred to nitrocellulose membranes, blocked in 4% milk in PBS and probed with a polyclonal antibody specific to the C-terminal 22 amino acids of APP (Zymed Laboratories; South San Francisco, CA). The blots were also probed with an anti-tubulin antibody (Sigma; St. Louis, MO) as a control. The blots were quantified using Kodak 1D Image Analysis software.

**Data analysis**

Statistica 6.0 (StatSoft, Inc.) was used to analyze the collected data. An α-level of 0.05 was used for all statistical significance tests. There were no gender×treatment interactions. Histological and biochemical data were analyzed using a one-way ANOVA with one between-subjects factor (group: control vs. PJ). Correlations between behavioral and biological variables were determined using the Pearson product–moment coefficient. To account for the pseudorandom nature of the distance to the escape platform from any given release point, swim path distance, escape latency, proximity to target and swim speed data were analyzed by averaging trials into blocks of 4 daily trials. These blocks were analyzed with two-way ANOVAs that included one between-subjects variable (group: control vs. PJ) and one within-subjects variable (test day). To avoid violating the assumptions of compound symmetry and sphericity that underlie univariate statistics for repeated measures factors with more than two levels (i.e., differences between levels of repeated measures must not be correlated across subjects), the reported p-values for every repeated-measures analysis reflect the Huynh–Feldt adjustment to the degrees of freedom. Probe trial data (time spent searching the probe quadrant and number of platform location crossings) for each phase and histological analyses were
Fig. 1. APP<sub>sw</sub> mice water maze performance. Each point represents the average of 4 daily trials. The bar graphs represent average performance (main effects) over each phase of testing. (A) Swim distance. PJ-treated mice learned the CUED task significantly more quickly (* treatment main effect \( p < 0.05 \); ** treatment × day interaction \( p < 0.02 \)). A similar trend was observed for the SPATIAL task, but the difference was not significant. (B) Swim speed. Both PJ-treated and control mice swam progressively faster during the CUED task, but the difference between the groups was not significant. Control mice swam progressively slower during the SPATIAL task, whereas PJ mice maintained a significantly higher rate of speed (* treatment main effect \( p < 0.04 \); ** treatment × day interaction \( p < 0.003 \)). (C) Escape latency. PJ-treated mice found the platform significantly more quickly in both the CUED task (* treatment main effect \( p < 0.02 \); ** treatment × day interaction \( p < 0.04 \)) and the SPATIAL task (* treatment main effect \( p < 0.02 \); ** treatment × day interaction \( p < 0.002 \)). (D) Proximity to platform. These data were gathered during the SPATIAL, but not the CUED, task. Mice treated with PJ spent more of each trial swimming closer to the target platform (* treatment main effect \( p < 0.05 \)).
used to indirectly assess water maze strategies (above chance levels, and probe trial performance improved across evidence of intact spatial memory, as probe trial search times were search time and platform annulus crossings). Both groups exhibited measures of spatial memory during the probe trials (i.e., quadrant on the SPATIAL task. The groups did not differ on putative proximity to target scores (proximity to target scores (510)), a measure that has been partially explained by the overall faster swim speeds of the PJ mice). Therefore, APPsw mice have demonstrated normal performance in the water maze test (Fig. 1). The PJ-treated APPsw mice learned the CUED (visible platform) task significantly faster than the control APPsw mice as assessed by both swim distance (p<0.05) and escape latency (p<0.02), although both groups had learned the task equally well by the final day of testing. Swim speed did not differ significantly between the groups on the Cued task. In the SPATIAL (hidden platform) task, PJ mice escaped the maze more quickly than controls (p<0.02), and swim distance data revealed a similar, but non-significant, learning curve. The discrepancy in significance between escape latency and swim distance data was partially explained by the overall faster swim speeds of the PJ mice during the SPATIAL trials (p<0.04). During the Cued task, both groups swam progressively faster on each day of testing. During the SPATIAL phase, control mice began to swim progressively slower on each test day, whereas PJ mice maintained a relatively constant swim speed across the 15 days. It has been shown that differences in search strategies can underlie differences in water maze performance (Jamas, 2004) and that different strategies are associated with different swimming speeds (Brodly and Holtzman, 2006). Therefore, “proximity to target”, a measure that has been used to indirectly assess water maze strategies (Magnusson, 1998), was analyzed. For each trial, the distance of the mouse to the center of the escape platform was measured every 0.055 s and summed until the trial ended. PJ mice had significantly lower cumulative proximity to target scores (p<0.05), suggesting better performance on the SPATIAL task. The groups did not differ on putative measures of spatial memory during the probe trials (i.e., quadrant search time and platform annulus crossings). Both groups exhibited evidence of intact spatial memory, as probe trial search times were above chance levels, and probe trial performance improved across all three probe trials for both groups (Fig. 2). There were no differences between wildtype mice fed PJ or vehicle in CUED or SPATIAL task performance or in swim speed (Fig. 3).

**PJ decreased Aβ and amyloid levels in the hippocampus**

APPsw mice develop large increases of Aβ in brain tissue at about the time that amyloid plaque deposition begins at 7–8 months of age (Hsiao et al., 1996). Thus, APPsw mice were treated with PJ or control treatment before plaque deposition (6 months of age) and assessed for Aβ levels and plaque load at a time point (12.5 months of age) after which plaques would be expected to be present. APPsw mice treated with PJ had significant reductions in Aβ deposition (53%; p<0.03; Fig. 4), thioflavine-S positive fibrillar Aβ (amyloid) deposition (50%; p<0.008; Fig. 4) and hippocampal soluble Aβ42 (51%; p<0.004; Fig. 5) as compared to control mice. Soluble and insoluble Aβ42:Aβ40 ratios were both significantly reduced in PJ-treated mice (p<0.04 and p<0.009, respectively). There were similar reductions in the dorsal cortex, although these decreases were not statistically significant. Levels of CAA in the dorsal cortex did not differ between groups. There was a non-significant, but consistent, trend for fewer dystrophic neurites associated with amyloid plaques in the dorsal cortex, hippocampus and corpus callosum. An analysis of amyloid plaque size revealed significantly smaller thioflavine-S positive plaques in the hippocampus, but not the dorsal cortex or corpus callosum, of the PJ group (p<0.004; data not shown).

**PJ treatment did not affect APP processing**

There may be several reasons why Aβ levels and Aβ deposition were lower in PJ-treated mice. One possibility is that PJ treatment resulted in decreased Aβ production via increased α-secretase and/or decreased β-secretase processing of APP. We found that there were no statistically significant differences between the groups in

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**Fig. 2. APPsw mice probe trial performance.** The bar graph shows the average of the three probe trials and represents the percent of probe trial search time spent searching each quadrant. Both groups demonstrated evidence of spatial memory, and there were no significant group differences.

**Fig. 3. Wildtype mice water maze performance.** There were no differences between wildtype mice fed PJ or vehicle in (A) CUED and SPATIAL task performance, (B) swim speed or probe trial performance (not shown).
Fig. 4. Brain Aβ load. Representative photomicrographs (2×) of brains from mice treated with control and PJ. Mice treated with PJ had significantly less Aβ (*p<0.03) and fibrillar (thioflavine-S+) Aβ (**p<0.008) in the hippocampus. There were similar reductions in the dorsal cortex, although these decreases were not statistically significant.
Fig. 5. Aβ and APP biochemistry. (A) Aβ ELISAs. Mice treated with PJ had significantly less hippocampal soluble Aβ_{42} (**p < 0.004) as compared to control mice. There were similar reductions in the dorsal cortex, but these decreases were not statistically significant. (B) Representative Western blots. Western blots were performed on 10 μg of total protein from cortical lysates of APPsw mice treated with PJ or control. (C) APP and APP C-terminal fragments. The percent optical density of the Western bands representing APP or APP fragments relative to tubulin is shown.
measurements of full-length APP, α-C terminal fragments (CTFs) or β-CTFs in the cortex (Fig. 5). These data suggest that the mechanism by which PJ influences behavior and amyloid load is not by altering APP processing or Aβ production.

Discussion

In this study, Tg2576/APP<sub>sw</sub> mice, commonly used as a model of Aβ deposition and associated AD-like pathology, were given pomegranate juice in their drinking water starting at 6 months of age. When their learning behavior was assessed at 1 year of age, PJ-treated mice exhibited improvements on cued and spatial learning tasks as well as faster overall swim speeds. Additionally, plaque load (both Aβ and fibrillar Aβ/amyloid) and soluble Aβ<sub>42</sub> were significantly reduced in the hippocampus. These results strongly suggest that pomegranates and/or their constituent substances should be further explored as potentially useful therapeutic agents.

PJ-treated APP<sub>sw</sub> mice learned both the cued and spatial components of the water maze more quickly than control mice. The superior performance of the PJ mice on the cued learning task suggests that there were some PJ-induced differences between the groups in general learning ability, swimming ability, visual acuity or motivation to escape the water, but these differences had dissipated by 20 trials over 5 days of CUED testing. Although the PJ mice found the platform more quickly during the SPATIAL trials, they were also swimming more quickly, which confounds interpretation of these data. A similar trend was seen for swim distance, a measure often used to control for the effect of swim speed on water maze performance. Swim distance, however, is not immune to the effects of swim speed. Given two trials in which the mice did not find the platform by the 60-second cut-off, the slower swimming mouse would actually swim a shorter distance for that trial. Even though both mice had performed equally poorly, the swim distance data would give the appearance of better performance to the slower mouse. The “cumulative proximity to target” measure is correlated with both escape latency and swim distance, but also partially controls for the search strategy used by the mouse. For example, consider two trials in which neither mouse found the platform, but one was searching in general vicinity of the platform, and the other was simply swimming around the perimeter of the pool. Even if they both swam the same total distance, the cumulative proximity score of the mouse that was searching the correct area would be smaller, indicating better performance. The fact that PJ-treated APP<sub>sw</sub> mice exhibited better performance in the SPATIAL task as assessed by the proximity score, in combination with the non-significant trend for better swim distance performance and the significant difference in swim speed, suggests that PJ-treated mice may have used more efficient search strategies than control mice. Performance on the probe trials, often reported as a putative measure of spatial memory, did not differ significantly between the groups. Both groups performed well above chance on all three probe trials, suggesting that PJ may not differ significantly between the groups. Both groups performed well above chance on all three probe trials, suggesting that PJ may have more of an effect on learning processes than on the facilitation of memory. Regardless of the effects of pomegranate juice on spatial learning, the effects on overall swim speed and performance during the CUED phase of testing were profound. We also assessed the effects of PJ versus vehicle in 3- to 5-month-old wildtype mice treated for 5 weeks. There was no difference in water maze performance between the PJ and vehicle-treated mice. This suggests that the effects of PJ in APP<sub>sw</sub> mice may be acting to slow or prevent a disease process. However, an experiment treating wildtype mice from 6 to 12.5 months of age would be required to make this conclusion.

PJ also reduced levels of soluble Aβ<sub>42</sub>, Aβ deposition and fibrillar Aβ/amyloid deposition in the hippocampi of APP<sub>sw</sub> mice. Other foods containing high levels of antioxidants and polyphenols have also been shown to reduce plaque deposition in APP<sub>sw</sub> mice. For example, both the essential fatty acid DHA (Calon et al., 2004; Lim et al., 2005) and chronic administration of the dietary antioxidant vitamin E (Morris et al., 2005) reduced plaque deposition in aged APP<sub>sw</sub> mice. Curcumin, a polyphenol found in the curry spice turmeric, was also shown to lower levels of oxidized proteins and plaque burden in APP<sub>sw</sub> mice (Lim et al., 2001). Another polyphenol found in green tea, epigallocatechin-3-gallate (EGCG), was recently shown to reduce production of Aβ and elevate levels of α-CTFs in vitro and to decrease levels of Aβ and plaque deposition in the brains of APP<sub>sw</sub> mice (Rezai-Zadeh et al., 2005). Tannic acid, also found in tea, has been shown to inhibit amyloid fibril formation in vitro (Ono et al., 2004). Interestingly, resveratrol, a polyphenol found in grapes and red wine, was shown to decrease levels of Aβ in vitro by increasing clearance, rather than inhibiting production, of Aβ (Marambaud et al., 2005). Thus, it appears that various naturally occurring polyphenols can decrease levels of Aβ and Aβ deposition in the brains of APP transgenic mice, possibly by inhibiting production, enhancing clearance or altering Aβ fibrillogenesis. Decreasing levels of Aβ may also ameliorate the tau pathology observed in the AD brain (Oddo et al., 2004). Recent data suggest that extracts from pomegranate husk contain β-secretase inhibitors (Kwak et al., 2005). In our experiments, analyses of APP CTFs suggest that PJ did not affect β-secretase cleavage of APP in the cortex. However, the pomegranate juice that we used did not contain material from the husk. It will be interesting for future studies to characterize the effects of pomegranate juice and husk on Aβ and APP processing, as well as its effects on production, clearance and fibrillogenesis in the brains of younger APP transgenic mice, both before and during the process of Aβ deposition. Whether PJ would decrease the amount of Aβ deposition, gliosis and neuritic dystrophy in older APP<sub>sw</sub> mice that already have abundant pathology will be of interest. If the effects of PJ are on Aβ fibrillogenesis or clearance, one might expect an effect since Aβ deposition continues over time even in old APP<sub>sw</sub> mice. In addition to effects on Aβ, it will be useful to determine if PJ affects other process such as inflammation and reactive oxygen species. Such studies will assist in understanding the mechanism of the effects of PJ.

Several other lines of research have shown beneficial effects of PJ intake on both humans and animal models of disease. A recent paper from our laboratory showed that dietary supplementation of pregnant mice with PJ resulted in significantly less brain damage to postnatal day 7 pups that were exposed to hypoxia–ischemia (Loren et al., 2005). Aβ is presumably not involved in the mechanism of protection in this neonatal model. This suggests that although the beneficial behavioral effects that we observed in adult APP<sub>sw</sub> mice may be due to an effect on Aβ, they may also be due to other mechanisms. Because recent evidence suggests that exercise may also reduce plaque load in a mouse model of AD (Adlard et al., 2005), it is interesting to speculate about whether increased swim speed in the PJ mice affected levels of Aβ in the brain.

Pomegranate juice has been shown to have a variety of actions in disease models, including reduced low-density lipoprotein (LDL) aggregation, oxidative stress (Aviram et al., 2000), serum angio-
tensin converting enzyme (ACE) and systolic blood pressure in hypertensive patients (Aviram and Dornfeld, 2001), and reduced LDL oxidation, atherosclerosis and systolic blood pressure in patients with carotid artery stenosis (Aviram et al., 2004). Numerous studies have found similar results in animals. PJ was found to reduce LDL aggregation and oxidative stress in apoE−/− mice (Aviram et al., 2000), and other studies have shown that PJ reduced atherosclerosis in hypercholesterolemic apoE−/− mice (Kaplan et al., 2001) and LDLR−/− mice (de Nigris et al., 2005) and improved cardiac lipid metabolism in a rat model of diabetes (Huang et al., 2005). It is possible that some of the vascular effects of PJ are via its effects on nitric oxide synthase in the vasculature (de Nigris et al., 2005). Perhaps surprisingly, levels of CAA were not affected by pomegranate consumption in this study. However, given the potential roles of cholesterol (Wellington, 2004) and vascular amyloidosis (Sadowski et al., 2004) in AD, these lines of research should be more fully explored. Pomegranates may have anti-cancer properties as well as anti-atherogenic properties (Albrecht et al., 2004; Burton, 2003; Kohno et al., 2004; Longtin, 2003; Mehta and Lansky, 2004), and it is likely that several polyphenols act synergistically to prevent tumor growth and contribute to its beneficial anti-atherogenic effects (Seeram et al., 2005). Interestingly, pomegranates also contain phytochemicals, and PJ has improved depressed behavior and bone properties in an ovariectomized mouse model of menopause (Mori-Okamoto et al., 2004). The vast number of compounds in PJ, along with the evidence that these compounds may act together in a synergistic fashion, suggests that isolated components of pomegranate may not be as effective as dietary supplementation with either the whole fruit or its juice.

This study is the first to show beneficial effects (both behavioral and neuropathological) of pomegranate juice in an animal model of AD. These data suggest that further studies to validate and determine the mechanism of these effects, as well as whether PJ can protect against AD in humans, may be warranted.

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