

Basic nutritional investigation

## Plum juice, but not dried plum powder, is effective in mitigating cognitive deficits in aged rats

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### Abstract

**Objective:** Normal aging in animals and humans is accompanied by a decline in cognitive performance that is thought to be due to the long-term effects of oxidative stress and inflammation on neurologic processes. Previous findings have suggested that protection against age-related cognitive declines may be achieved by increasing the dietary intake of fruits and vegetables, especially those that are high in antioxidant activity, such as blueberries and strawberries. The objective of this study was to investigate supplementation with *Prunus domestica* L. in mitigating age-related deficits in cognitive function.

**Methods:** We investigated the effects of supplementation with *P. domestica* L., consumed as a 2% dried plum (i.e., prune) powder or 100% plum juice for 8 wk, in mitigating age-related deficits in cognitive function in aged Fischer 344 rats.

**Results:** Rats that drank plum juice from 19 to 21 mo of age had improved working memory in the Morris water maze, whereas rats fed dried plum powder were not different from the control group, possibly due to the smaller quantity of phenolics consumed in the powder group compared with the juice group.

**Conclusion:** These results are discussed in relation to the amount and type of phenolics present in the plum products and in relation to other dietary intervention studies in which cognitive benefits have been reported. © 2009 Published by Elsevier Inc.

### Keywords:

Polyphenolics; Hydroxycinnamates; Antioxidant; Spatial memory and learning; Prunes; Anthocyanins; Chlorogenic

### Introduction

Normal neurologic aging in animals and humans is accompanied by declines in cognitive performance [1–4], thought to be due to an increased susceptibility of the brain to the long-term effects of oxidative stress and inflammation (for reviews, see Joseph et al. [5] and Shukitt-Hale [6]). As the proportion of elderly in our population increases, so will the prevalence of cognitive impairment due to normal aging and to neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [7–9]. To improve the quality of life

for the elderly and to mitigate the social and economic burdens of increased life expectancy, it is critical to devise strategies that will slow the decline in cognitive performance arising from oxidative stress and inflammation. Previous findings have suggested that age-related cognitive declines may be mitigated by the consumption of fruits and vegetables, especially the darker-colored ones, that contain phytochemicals with high in vitro antioxidant and anti-inflammatory activity (for reviews, see Joseph et al. [5], Cao et al. [10], Wang et al. [11], and Shukitt-Hale et al. [12]).

Phenolic phytochemicals, which are abundant in fruit crops, possess a myriad of biological activities, including potent in vitro antioxidant activity [13]. Indeed, dietary phenolics have been reported to upregulate antioxidant pathways, providing greater benefit to antioxidant protection systems than simply their in vivo concentration and

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redox capacity. Phenolics may help to downregulate inflammatory pathways through their inhibitory effect on nuclear factor- $\kappa$  and enzymes leading to inflammatory events. Phenolics can affect gene transcription, cell-cycling events, and apoptosis. As a result, phenolics provide benefits to cardiovascular health, cancer chemoprotection in various models of inflammation, and, of particular interest to the present study, in neuroprotection (for review, see Stevenson and Hurst [13]).

Our laboratory has shown that when the diets of aged (>19-mo-old) rats were supplemented for 8 wk with Concord grape juice as the sole source of liquid, the neurochemical and behavioral changes that characterize brain aging in these rats was reversed [14]. Eight weeks of dietary supplementation to aged rats (19–21 mo old) with dark-colored fruits and vegetables, including extracts of spinach, strawberry, or blueberry, in an AIN-93 diet or blueberry in a chow-based diet, were also effective in reversing several parameters of neuronal function (e.g., deficits in cell communication such as dopamine release) [15,16] and age-related motor and cognitive deficits [15–19]. Other berry fruits (i.e., cranberries and blackcurrants) were also effective in this model [12], suggesting that phytochemicals present in antioxidant-rich foods [20] might be effective in forestalling functional age-related deficits.

California dried plums (i.e., prunes, *Prunus domestica* L) are produced from a cultivar referred to by several names including French, Petite, d'Agen, Petite d'Agen, and d'Ente [21]. To produce prunes, plums are dehydrated in air at 85–90°C for 18 h to reduce fruit water content from 87% to approximately 33% [20]. Prunes (i.e., dried plums) may be processed further into prune juice, purée, or other prune products [22]. In a study of more than 100 common foods in the United States, plums and prunes were found to have a high total antioxidant capacity when measured by the oxygen radical absorbing capacity (ORAC) assay [20]. The total (lipophilic and hydrophilic) ORAC of fresh plums was reported as 59.2  $\mu$ mol Trolox equivalents (TE)/g of edible portion [20]. Prunes were reported to have a total (lipophilic and hydrophilic) ORAC of 85.8  $\mu$ mol TE/g of edible portion, which was nearly three times higher than the other dried fruits such as figs, raisins, and dates [20]. Recently dried plums have been recognized as a healthy food, due to their laxative action, ability to delay glucose absorption, and as preventive agents against chronic diseases, such as heart disease, cancer, and osteoporosis [22].

The concentration of total phenolics in fresh prune-making plums is approximately 1.10 mg/g of fresh edible portion, based on high-performance liquid chromatographic analysis [21] which is approximately equivalent to 8.47 mg/g dry weight (DW) total phenolics based on a fruit moisture content of 87%. The phenolics in fresh prune-making plums include hydroxycinnamates (84–90%), flavonols (2–3%), the flavan-3-ol catechin (4–8%), and anthocyanins (4–9%) [21]. During the drying of plums to make prunes, phenolics are degraded due to the action of

polyphenolic oxidase and non-enzymic Maillard reactions [23]. Donovan et al. [21] reported that half of the flavonols and hydroxycinnamates and all of the anthocyanins and flavan-3-ols were lost during commercial drying of plums. The phenolic concentration of prunes is approximately 1.84 mg/g of the fresh edible portion [21,22] or 2.67 mg/g DW total phenolics, based on a prune moisture content of 33%. Plums and prunes, which are notable for their abundance and variety of hydroxycinnamate esters, contain chlorogenic acid (i.e., 5-*O*-caffeoylquinic acid) isomers including 3-*O*-caffeoylquinic acid (neochlorogenic acid) and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid) [20,21,24,25].

Given that plums and dried plums contain an abundant variety of hydroxycinnamate esters, although dried plums are essentially devoid of flavonoids, we compared the effects of supplementation with these two products in our in vivo model that examined age-related deficits in cognitive function [15]. The two plum products were tested in comparable but separate studies, which examined aged Fischer 344 rats, where cognitive testing was conducted after 8 wk of supplementation with 100% plum juice or 2% dried plum powder diet.

## Materials and methods

### Animal care

Nineteen-month-old male Fischer 344 rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were employed. Fischer 344 rats have been shown to exhibit neurologic decrements by 15 mo of age in age-valid tests [4]. Rats were individually housed in stainless-steel mesh-suspended cages, maintained on a 12-h light/dark cycle, and were provided with food and water (by automatic watering) ad libitum, as specified in the two studies. Food intake was also measured during the course of the study. Animal weights were recorded at several time points and rats were examined daily for clinical signs of disease. Animals were used in compliance with all applicable laws and regulations and with the principles expressed in the National Institutes of Health, United States public health service, Guide for the Care and Use of Laboratory Animals. Both studies were approved by the animal care and use committee of the U.S. Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University.

### Dried plum powder study

Thirty 19-mo-old rats were used in this study. After a 2-wk period of acclimation to the facility, rats were weight-matched and then randomly assigned to the control group or the dried plum powder group ( $n = 15$ /group). During the course of the study, one rat in the control group died and one in the dried plum group was removed from the study because of extensive weight loss due to a pituitary tumor.

The dried plum diet was prepared at Harlan Teklad (Madison, WI, USA) by adding dried plum powder to the control diet, which was a modification of the NIH-31 diet, i.e., the amount of corn in the control diet was adjusted to compensate for the added volume of the dried plums. The control NIH-31 diet was the same as used in previous studies where blueberries were found to be beneficial in mitigating brain aging [16,19,26,27]. The dried plum powder (available only as a commercial industrial food ingredient) was composed of 99% dried plums (Petite d'Agen variety) and 1% calcium stearate (Taylor Bros. Farms, Inc., Yuba City, CA, USA) and was added to the NIH-31 diet at 20 g/kg. The total phenolic concentration [28] of the dried plum powder was 6.86 mg gallic acid equivalents (GAE)/g DW of powder, and no anthocyanins were detected in the powder. Water-soluble ORAC [10] was 198  $\mu\text{mol TE/g}$  of DW powder. Rats on the control or dried plum powder-supplemented diet consumed food and water ad libitum for 8 wk before cognitive testing was conducted at 21 mo of age.

#### *Plum juice study*

Twenty-eight 19-mo-old rats were used in this study. Once the rats were weight-matched, they were randomly assigned to the control group (water,  $n = 13$ ) or the plum juice group ( $n = 15$ ). Rats consumed an NIH-31 diet (Harlan Teklad, product 7017) and fluids (water or plum juice) ad libitum. During the course of the study, one rat in the plum juice group developed visible tumors in both eyes and was removed from the study.

Plum phenolics were provided in the form of reconstituted single-strength juice made from concentrate (Sun-sweet Growers, Inc., Yuba City, CA, USA) produced from mature plums of the Petite d'Agen variety. The plum juice was prepared as a mixture of 20.4% (v/v) plum juice concentrate and 79.6% water, which yielded a 14.3° Brix solution. Total phenolic concentration in the reconstituted plum juice was 6.29 mg GAE/g DW of juice (1264 mg GAE/L) [28] and the anthocyanin content was 0.009 mg cyanidin-3-glucoside equivalents (C3GE)/g DW of juice (1.80 mg C3GE/L) measured by high-performance liquid chromatography. Water-soluble ORAC [10] of the plum juice was 156  $\mu\text{mol TE/g}$  of DW juice or 31.3 mmol TE/L. Refrigerated concentrate was used to make fresh single-strength plum juice three times per week. Juice or water was provided to the rats in small glass jars secured to the cages to prevent spillage. Jars and fluids were replaced in the cages every other day. The rats were maintained on the control (water) or plum juice as their only source of fluids for 8–9 wk before cognitive testing at 21 mo.

#### *Antioxidant measurements*

Antioxidant capacity of plum products and serum obtained from rats at euthanasia after 9–10 wk of supplementa-

tion were measured using the ORAC assay [10]. ORAC was carried out using fluorescein as the fluorescent indicator of antioxidant capacity [29]. Fruit ORAC was carried out on extracts dissolved in water. ORAC was measured on serum after protein was removed using perchloric acid [29].

#### *Cognitive testing*

The Morris water maze (MWM) [30] is an age-sensitive [1,4,31] learning paradigm that requires the rat to use spatial learning to find a hidden platform (10 cm in diameter) submerged 2 cm below the water's surface in a circular pool and to remember the platform location from the previous trial. The pool is 134 cm in diameter by 50 cm in height and maintained at 23°C. The rat uses distal cues to effectively locate the platform; accurate navigation is rewarded with escape from the water onto the platform.

To assess working memory (i.e., short-term memory or the ability to remember information over a brief period) in the MWM [1,30], daily sessions were performed for 4 consecutive days during the eighth or ninth week of dietary intervention, with a morning and an afternoon session, two trials in each session, and a 10-min interval between the two trials. Rats were tested in a random manner, except that testing was alternated between rats on the control and plum-supplemented diets. To begin each trial, a rat was gently immersed in the water at one of four randomized start locations. The rat was allowed 120 s to swim and find the platform; if the rat failed to locate it within this time, it was guided to the platform. Once on the platform, the rat remained there for 15 s. After trial 1, which was the acquisition trial, to assess reference memory, the rat was returned to its cage for 10 min until trial 2. Trial 2, the retrieval trial, assessed working memory by using the same platform location and start position as trial 1. Performances were videotaped and analyzed with image tracking software (HVS Image, Buckingham, United Kingdom) and allowed the measurement of the latency to find the platform (seconds), total path length (centimeters), and swimming speed (centimeters per second; latency/path length). For a more detailed description of the MWM and the paradigm used, see Shukitt-Hale et al. [4].

#### *Statistical analyses*

Because the diet (NIH-31) and source of liquid (water) for the control groups in the studies on both plum products was the same, and because the two control groups were not statistically different in their MWM latency and distance measurements, results for the two control groups were combined ( $n = 27$ ) for statistical analyses on these behavioral measurements. For each MWM behavioral measurement, a between-subjects analysis of variance model was used to statistically compare the three groups (control, dried plum powder, plum juice) using Systat (SPSS, Inc., Chicago, IL, USA). Days or trials were included in the model when

appropriate, as a within-subjects variable. Post hoc comparisons to examine differences among groups were performed using Fisher's least significant difference post hoc analysis. To analyze working memory, separate *t* tests were conducted for each group between the trial 1 and trial 2 latencies and distances.

## Results

There was no difference in food intake between the control and dried plum powder diet groups; final body weight after 9–10 wk of feeding was not different (Table 1). However, in the plum juice study, rats receiving plum juice ate significantly less solid food than the control rats ( $F_{1,25} = 21.03$ ,  $P < 0.01$ ), even though body weight and fluid intake between the two groups was not different (Table 1). Rats receiving dried plum powder consumed  $3.30 \pm 0.10$  mg GAE/d of phenolics (mean  $\pm$  SEM) due to dried plum supplementation. Rats receiving plum juice consumed  $30.3 \pm 1.12$  mg GAE/d of phenolics and  $0.043 \pm 0.002$  mg C3GE/d of anthocyanins due to plum juice supplementation. Note that the rats that drank the plum juice received more than nine times more total phenolics daily than the rats that ate the dried plum powder, because rats drank 100% plum juice compared with only 2% dried plum in the diet.

Compared with the control group, rats receiving plum juice showed improved performance in the MWM, whereas the rats supplemented with dried plum powder showed no change in their MWM performance. Over the 4 d of testing, trial 2 performance, which assessed working memory, was significantly better in the rats receiving plum juice compared with the control or dried plum groups. Improved performance by rats in the plum juice group was observed in measurements of latency to find the platform ( $F_{2,52} = 6.04$ ,  $P < 0.01$ ; Fig. 1) and distance traveled ( $F_{2,52} = 4.25$ ,  $P < 0.05$ ; Fig. 2). The performance of rats in trial 1, which assessed reference memory, i.e., long-term memory or the ability of rats to remember the task, was not different among the three diet groups (dried plum powder, plum juice, or control; Figs. 1 and 2).

Table 1

Individual rat body weight at the start and finish of the studies and mean daily intake of solid and liquid food over the 9- to 10-wk study\*

Supplementation	Body weight (g)		Fluid intake (mL/d)	Food intake (g/d)
	Week 0	Week 9/10		
Control diet	450 $\pm$ 7	447 $\pm$ 5	N/A	22.7 $\pm$ 0.7
2% dried plum diet	445 $\pm$ 6	439 $\pm$ 6	N/A	24.1 $\pm$ 0.7
Water	414 $\pm$ 10	415 $\pm$ 13	27.0 $\pm$ 1.5	21.5 $\pm$ 1.1
100% plum juice	416 $\pm$ 7	395 $\pm$ 9	24.0 $\pm$ 0.9	16.0 $\pm$ 0.6 <sup>†</sup>

N/A, not applicable

\* Values are means  $\pm$  SEMs.

<sup>†</sup>  $P < 0.01$  compared versus water control group.

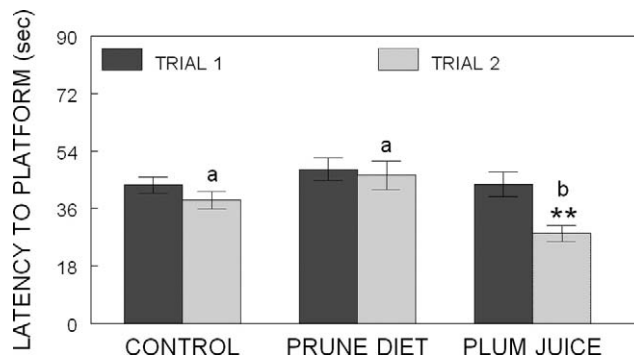


Fig. 1. Morris water maze performance assessed as latency (mean  $\pm$  SEM) to find the hidden platform over the 4 d of testing. Means not sharing a common letter are significantly different from each other ( $P < 0.05$ , Fisher's least significant difference test). There was a difference (i.e., an improvement) between trial 1 and trial 2 performance for the plum juice group (\*\* $P < 0.01$ , *t* test), meaning that the plum juice group had improved working memory. This improvement was not seen in the control or the prune diet rats.

Cognitive improvement (i.e., improved working memory) in the rats receiving plum juice was also apparent when separate *t* tests were conducted between the trial 1 and trial 2 latencies and distances. Between trials 1 and 2 there was a significant reduction in latency to find the platform ( $t_{13} = 3.15$ ,  $P < 0.01$ ; Fig. 1) and in the total distance traveled ( $t_{13} = 3.09$ ,  $P < 0.01$ ; Fig. 2). Because trial 2 measurements of latency and distance were significantly less than in trial 1, the rats fed plum juice demonstrated one-trial learning, even when there was a 10-min delay between the two trials. This one-trial learning and improvement in working memory was not found in the control or dried plum groups (Figs. 1 and 2).

When ORAC [20,29] was used to determine whether the dietary treatments affected the antioxidant capacity of perchloric acid deproteinized blood serum, rats receiving plum

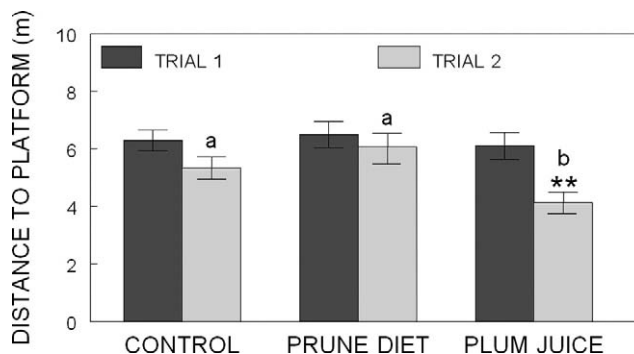


Fig. 2. Morris water maze performance assessed as distance (mean  $\pm$  SEM) to find the hidden platform over the 4 d of testing. Means not sharing a common letter are significantly different from each other ( $P < 0.05$ , Fisher's least significant difference test). There was a difference (i.e., an improvement in working memory) between trial 1 and trial 2 performance for the plum juice group (\*\* $P < 0.01$ , *t* test). This improvement was not seen in the control or the prune diet rats.

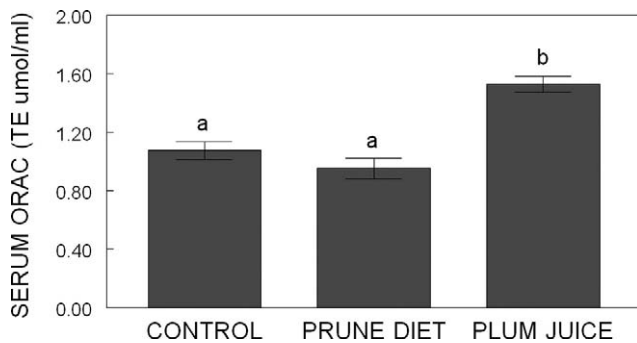


Fig. 3. Serum ORAC assessed as TE (micromoles per milliliter) in deproteinized samples (using perchloric acid; mean  $\pm$  SEM). Means not sharing a common letter are significantly different from each other ( $P < 0.01$ , Fisher's least significant difference test). ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents.

juice showed a significantly higher serum ORAC compared with serum obtained from rats in the control and dried plum groups ( $F_{2,41} = 12.65$ ,  $P < 0.001$ ; Fig. 3).

## Discussion

The benefit of plum juice consumption and the lack of benefit of dried plum powder consumption in the MWM performance are interesting in relation to the different dose and form of plum products consumed by rats reported in this study. Rats in the dried plum powder group consumed substantially less total phenolics (3.30 mg GAE per rat per day) than rats consuming plum juice (30.3 mg GAE mg per rat per day). If we had fed more than 2% of the dried plum powder to the rats, we also might have seen positive effects with this treatment. Although it is possible that the higher dose of phenolics consumed by the plum juice group may have been solely responsible in the differential benefits observed, it may be that the form of the plum product also contributed to this difference. Plum juice phenolics are mainly (84–90%) hydroxycinnamates; however, Donovan et al. [21] reported that fresh Petite d'Agen plums also contain flavonoids, including flavonols (2–3% of total phenolics), flavanols (i.e., catechins, 4–8%), and anthocyanins (4–9%). However, after drying to produce prunes, essentially no flavonoids remained in Petite d'Agen plums; anthocyanins and catechins were no longer present and flavonols were present at about 2% of the total phenolics [21]. Therefore the phenolic composition of prunes is essentially only hydroxycinnamate esters (>90%), primarily various chlorogenic acid isomers. The absence of flavonoids in the dried plum powder may have also contributed to the lack of effect in dried plum powder-fed rats in the MWM, because flavonoids typically possess greater bioactivity than hydroxycinnamates. Compared with hydroxycinnamates, flavonoids are more potent antioxidants [32] and are also more protective in models of vascular oxidative stress, inflammation [33], and atherosclerosis [34]. Furthermore, another

investigation using a similar dried plum powder found that the concentration of neochlorogenic acid and chlorogenic acid in the dried plum powder was considerably less than that reported for dried plums [35]. In their study, the dried plum powder contained <5% neochlorogenic acid and <10% chlorogenic acid of that reported for dried plums, most likely due to the high temperatures used to produce the dried plum powder [35]. Therefore, it is possible that prunes alone or perhaps prune juice could have had a beneficial effect, if tested in this study.

Blueberries, strawberries, and grapes, which are rich in phytochemicals, particularly phenolics, have been reported to provide cognitive benefits in the MWM model and, in some cases, in psychomotor performance [14,15]. Blueberries are rich in anthocyanin flavonoids that are associated with health benefits [36]. Strawberries are distinctive in that they contain health-beneficial ellagitannins. Red grapes, blueberries, and strawberries contain varying concentrations of flavonols, flavan-3-ols (catechins), proanthocyanidins, anthocyanins, and the non-flavonoid hydroxycinnamic acid ester, chlorogenic acid [37]. Flavonoids like these appear to exert health-protective effects through antioxidant mechanisms and, possibly more importantly, through direct effects on cellular processes including anti-inflammatory pathways, gene transcription and cell cycling, and apoptosis (for review, see Stevenson and Hurst [13]).

In previous experiments rats fed blueberry-, strawberry-, and 10% Concord grape juice-supplemented diets consumed 4.4 to 6.4 times less total phenolics than rats fed plum juice, even though all four of these dietary treatments provided benefits in MWM performance (Table 2). This would suggest that the flavonoids present in blueberry, strawberry, and 10% grape juice might be more effective in improving MWM performance, because MWM benefits were achieved at a lower total phenolic concentration than in the plum juice group. Alternatively, there could be a threshold for effect, above which more phenolics are not beneficial. It also may suggest that chlorogenic acid isomers contained in the plum juice were less potent in affecting MWM performance. Although Donovan et al. [21] reported that 4–9% of fresh plum phenolics may be anthocyanins, the proportion of anthocyanins in the daily dosage of plum juice in the present study was only 0.15% of the total plum phenolics (Table 2), suggesting that anthocyanins, and likely other flavonoids, may have been lost during the production of the plum juice concentrate. According to Table 2, rats consuming the dried plum powder received less total phenolics compared with rats in other studies, so it is also possible that, if the dose had been doubled, that the dried plum powder would have shown some beneficial effects.

In addition to considering the relative bioactivity of flavonoids and non-flavonoids in MWM effects, it is also important to consider their relative bioavailability. A recent review on the bioavailability of different phenolics classes has indicated that the in vivo concentration of native hydroxycinnamates is significantly greater than that of native

Table 2  
Estimated daily intake of total phenolics, anthocyanins, and ORAC equivalents from fruit products and their effects on MWM performance

Fruit product	Dose (%)	MWM latency*	MWM distance <sup>†</sup>	Phenolics <sup>‡</sup>	Anthocyanins <sup>§</sup>	ORAC <sup>  </sup>	Reference
Plum juice	100	**	**	30.3	0.043	750	Present study
Dried plum powder	2	NS	NS	3.30	0	94.8	Present study
Blueberry powder	1.86	**	**	4.72	0.98	152	Joseph et al. [15] <sup>¶</sup>
Strawberry powder	1.48	#	#	6.90	0.45	NA	Joseph et al. [15] <sup>¶</sup>
Grape juice	10	#	#	6.55	0.803	84.1	Shukitt-Hale et al. [14]
Grape juice	50	NS	NS	30.6	4.35	457	Shukitt-Hale et al. [14]

MWM, Morris water maze; ORAC, oxygen radical absorbing capacity

\* Latency to find platform.

<sup>†</sup> Distance traveled to find platform.

<sup>‡</sup> Milligrams of gallic acid equivalents consumed per rat per day (based on drinking 24 mL of juice/d or eating 25 g of diet/d).

<sup>§</sup> Milligrams of cyanidin-3-glucoside equivalents consumed per rat per day (based on drinking 24 mL of juice/d or eating 25 g of diet/d).

<sup>||</sup> Micromoles of Trolox equivalents consumed per rat per day (based on drinking 24 mL of juice/d or eating 25 g of diet/d).

<sup>¶</sup> Phenolic and anthocyanin levels were not reported but were calculated for this table from fruit used in this study.

#  $P < 0.05$  versus control.

\*\*  $P < 0.01$  versus control.

flavonoids, and that hydroxycinnamates are not extensively metabolized by phase II metabolism [13]. This may be supported by our current and previous studies, where 50% Concord grape juice, which contained flavonoids, provided approximately the same amount of total phenolics (30.6 mg GAE per rat per day) as plum juice (30.3 mg GAE per rat per day); however, the 50% grape juice did not affect MWM performance, whereas plum juice was effective (Table 2). Interestingly, 10% Concord grape juice improved MWM performance, whereas 50% grape juice did not. It may be that the higher level of grape phenolic intake induced phase II metabolism of flavonoids, giving rise to higher levels of their conjugates, which were not effective in modulating MWM performance. The higher dosage of plum hydroxycinnamates needed to observe an effect in the MWM compared with other fruit (Table 2) suggests that the bioactivity of hydroxycinnamates in the MWM is low, despite their greater bioavailability. The bioavailability of the plum juice phenolics was apparent by the greater serum ORAC compared with the control group (Fig. 3).

The quantity and quality of fruit phenolic should also be considered in relation to the high-molecular-weight phenolics that arise as a result of enzymic and non-enzymic processes during fruit processing and storage, including during plum drying and prune storage [23]. During drying, cellular compartmentation is destroyed, allowing the enzyme polyphenol oxidase to react with chlorogenic acid isomers to form reactive quinones that can oxidize anthocyanins, giving rise over time to anthocyanin-containing phenolic heteropolymers of increasing molecular weight [23]. Also Maillard-type non-enzymic reactions between phenolic compounds and protein moieties result in the formation over time of high-molecular-weight mixed phenolics [23]. Although little is known about their bioavailability, it is generally assumed that these processing-derived high-molecular-weight compounds are very poorly absorbed, if at all. It is interesting to note that the dried plum powder has a relatively high ORAC value, a measurement of antioxi-

dant capacity [35]. However, the ORAC and other assays are tests of how compounds behave in vitro, but how the compound will behave in vivo depends on other factors such as bioavailability and bioactivity. Therefore one cannot assume, if a compound has a high ORAC, that it will necessarily have positive effects in a biological system.

## Conclusion

Taken together the results of the present study show that plum juice had a beneficial effect in reversing age-related declines in cognitive behavior, whereas the dried plum powder, which is derived from the same plums as the plum juice, did not have a positive effect on memory. As discussed, the reasons for this difference could be related to the quantity of phenolics consumed, the type and amount of different phenolics present in the fruit, the effects processing has on these phenolic compounds, and their bioactivity.

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## References

- [1] Brandeis R, Brandys Y, Yehuda S. The use of the Morris water maze in the study of memory and learning. *Int J Neurosci* 1989;48:29–69.
- [2] Ingram DK, Jucker M, Spangler E. Behavioral manifestations of aging. In: Capen CC, editor. *Pathobiology of the aging rat*. Washington, DC: ILSI; 1994, p. 149–70.
- [3] Keller JN. Age-related neuropathology, cognitive decline, and Alzheimer's disease. *Ageing Res Rev* 2006;5:1–13.
- [4] Shukitt-Hale B, Mouzakis G, Joseph JA. Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Exp Gerontol* 1998;33:615–24.

- [5] Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005; 81(suppl):313S–6.
- [6] Shukitt-Hale B. The effects of aging and oxidative stress on psychomotor and cognitive behavior. *Age* 1999;22:9–17.
- [7] Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiol Aging* 2002;23:719–35.
- [8] Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, et al. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA* 1989;262:2551–6.
- [9] Nicita-Mauro V. Parkinson's disease, Parkinsonism and aging. *Arch Gerontol Geriatr* 2002;8(suppl):225–38.
- [10] Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. *J Agric Food Chem* 1996;44:3426–31.
- [11] Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. *J Agric Food Chem* 1996;44:701–5.
- [12] Shukitt-Hale B, Galli R, Meterko V, Carey A, Bielinski D, McGhie T, Joseph JA. Dietary supplementation with fruit polyphenolics ameliorates age-related deficits in behavior and neuronal markers of inflammation and oxidative stress. *Age* 2005;27:49–57.
- [13] Stevenson DE, Hurst RD. Polyphenolic phytochemicals—just antioxidants or much more? *Cell Mol Life Sci* 2007;64:2900–16.
- [14] Shukitt-Hale B, Carey A, Simon L, Mark DA, Joseph JA. The effects of Concord grape juice on cognitive and motor deficits in aging. *Nutrition* 2006;22:295–302.
- [15] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* 1999;19: 8114–21.
- [16] Youdim KA, Shukitt-Hale B, Martin A, Wang H, Denisova N, Bickford PC, et al. Short-term dietary supplementation of blueberry polyphenolics: beneficial effects on aging brain performance and peripheral tissue function. *Nutr Neurosci* 2000;3:383–97.
- [17] Bickford PC, Shukitt-Hale B, Joseph J. Effects of aging on cerebellar noradrenergic function and motor learning: nutritional interventions. *Mech Ageing Dev* 1999;111:141–54.
- [18] Bickford PC, Gould T, Briederick L, Chadman K, Pollock A, Young D, et al. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. *Brain Res* 2000;866:211–7.
- [19] Goyarzu P, Malin DH, Lau FC, Tagliatalata G, Moon WD, Jennings R, et al. Blueberry supplemented diet: effects on object recognition memory and nuclear factor-kappa B levels in aged rats. *Nutr Neurosci* 2004;7:75–83.
- [20] Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem* 2004;52:4026–37.
- [21] Donovan JL, Meyer AS, Waterhouse AL. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *J Agric Food Chem* 1998;46:1247–52.
- [22] Stacewicz-Sapuntzakis M, Bowen PE, Hussain EA, Damayanti-Wood BI, Farnsworth NR. Chemical composition and potential health effects of prunes: a functional food? *Crit Rev Food Sci Nutr* 2001; 41:251–86.
- [23] Del Caro A, Piga A, Pinna I, Fenu PM, Agabbio M. Effect of drying conditions and storage period on polyphenolic content, antioxidant capacity, and ascorbic acid of prunes. *J Agric Food Chem* 2004;52: 4780–4.
- [24] Fang N, Yu S, Prior RL. LC/MS/MS characterization of phenolic constituents in dried plums. *J Agric Food Chem* 2002;50:3579–85.
- [25] Kayano S, Yamada NF, Suzuki T, Ikami T, Shioaki K, Kikuzaki H, et al. Quantitative evaluation of antioxidant components in prunes (*Prunus domestica* L.). *J Agric Food Chem* 2003;51:1480–5.
- [26] Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee HG, Smith MA, et al. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. *Nutr Neurosci* 2004;7:309–16.
- [27] Joseph JA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci* 2003;6:153–62.
- [28] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965;16:144–58.
- [29] Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem* 2001;49:4619–26.
- [30] Morris R. Development of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 1984;11:47–60.
- [31] Ingram DK, Jucker M, Spangler EL. Behavioral manifestations of aging. In: Mohr U, Cungworth DL, Capen CC, editors. *Pathobiology of the aging rat*. ILSI: Washington. 1994;pp 149–70.
- [32] Proteggente AR, Saija A, De Pasquale A, Rice-Evans CA. The compositional characterisation and antioxidant activity of fresh juices from Sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. *Free Radic Res* 2003;37:681–7.
- [33] Youdim KA, McDonald J, Kalt W, Joseph JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults (small star, filled). *J Nutr Biochem* 2002;13:282–8.
- [34] Auger C, Laurent N, Laurent C, Besancon P, Caporiccio B, Teissedre PL, et al. Hydroxycinnamic acids do not prevent aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters. *Life Sci* 2004; 74:2365–77.
- [35] Yang Y, Gallaher DD. Effect of dried plums on colon cancer risk factors in rats. *Nutr Cancer* 2005;53:117–25.
- [36] Kalt W, Joseph JA, Shukitt-Hale B. Blueberries and human health: a review of current research. *J Am Pomol Soc* 2007;61:151–60.
- [37] Macheix J-J, Fleuriet A, Billot J. *Fruit phenolics*. Boca Raton: CRC Press; 1990.