Grape Powder Supplementation Prevents Oxidative Stress–Induced Anxiety-Like Behavior, Memory Impairment, and High Blood Pressure in Rats

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Abstract

We examined whether or not grape powder treatment ameliorates oxidative stress–induced anxiety-like behavior, memory impairment, and hypertension in rats. Oxidative stress in Sprague-Dawley rats was produced by using L-buthionine-(S,R)-sulfoximine (BSO). Four groups of rats were used: (1) control (C; injected with vehicle and provided with tap water), (2) grape powder–treated (GP; injected with vehicle and provided for 3 wk with 15 g/L grape powder dissolved in tap water), (3) BSO–treated (injected with BSO [300 mg/kg body weight], i.p. for 7 d and provided with tap water), and (4) BSO plus grape powder–treated (GP+BSO; injected with BSO and provided with grape powder–treated tap water). Anxiety-like behavior was significantly greater in BSO rats compared with C or GP rats (P < 0.05). Grape powder attenuated BSO-induced anxiety-like behavior in GP+BSO rats. BSO rats made significantly more errors in both short- and long-term memory tests compared with C or GP rats (P < 0.05), which was prevented in GP+BSO rats. Systolic and diastolic blood pressure was significantly greater in BSO rats compared with C or GP rats (P < 0.05), whereas grape powder prevented high blood pressure in GP+BSO rats. Furthermore, brain extracellular signal-regulated kinase-1/2 (ERK-1/2) was activated (P < 0.05), whereas levels of glyoxalase-1 (GLO-1), glutathione reductase-1 (GSR-1), calcium/calmodulin-dependent protein kinase type IV (CAMK-IV), cAMP response element–binding protein (CREB), and brain-derived neurotrophic factor (BDNF) were significantly less (P < 0.05) in BSO but not in GP+BSO rats compared with C or GP rats. We suggest that by regulating brain ERK-1/2, GLO-1, GSR-1, CAMK-IV, CREB, and BDNF levels, grape powder prevents oxidative stress–induced anxiety, memory impairment, and hypertension in rats. J. Nutr. 143: 835–842, 2013.

Introduction

There is growing evidence of occurrence of cognitive dysfunction and presence of anxiety disorders in people suffering from hypertension (1,2) and of the occurrence of hypertension and cognitive impairment in people suffering from anxiety disorder (3–6). To define the nature and cause(s) of this comorbidity and to identify effective treatments, animal models that test the intersection of these conditions are needed.

Recently, using our animal model of oxidative stress, we reported a causal role of oxidative stress in anxiety-like behavior (7) and high blood pressure (8) in rats. Reports from other laboratories are in agreement with our findings (9–15). Basically, our studies demonstrate that direct induction of oxidative stress via L-buthionine-(S,R)-sulfoximine (BSO)4 treatment increases anxiety-like behavior in rats and treatment with the antioxidant tempol reduces oxidative stress and attenuates anxiety-like behavior of rats, suggesting a causal role of oxidative stress in anxiety-like behavior (7). Furthermore, we have shown that acute sleep deprivation increases oxidative stress and causes anxiety-like behavior (16) and memory impairment in rats (17). These studies suggest similar behavioral consequences of direct versus indirect induction of oxidative stress, i.e., anxiety-like behavior and cognitive impairment. Although our observations with tempol are significant, synthetic antioxidant compounds

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have limitations. For example, tempol is an unstable compound with unknown side effects, which limit its usefulness in the clinic. Therefore, we focused on natural products with known antioxidant properties.

The potential health benefits of grapes have been known for a long time (18). Although most studies link health benefits of grapes to cardiovascular activities (19), many other types of responses are conceivable. Although the antioxidant properties of grapes are emphasized (resveratrol, a polyphenol found in grape seeds and a constituent of red wine (20,21), has received considerable attention and been shown to have antioxidant properties (22–24)), grape powder contains other important antioxidant components as well. In fact, a phytochemical analysis of grapes has revealed hundreds of constituents that may be capable of mediating biological responses. The Natural Product Alert (NAPRALERT) database has reported >1600 compounds (19) representing >30 chemical classes (18). Harnessing the natural antioxidant properties of grapes to mimic anxiolytic effects of tempol in our oxidative stress model of anxiety would be important because they offer the potential for a safe intervention without any harmful side effects of synthetic/pharmacologic interventions.

Although several studies have reported beneficial effects of grapes on cognition and anxiety (25–27), none have investigated their effect in the brain after direct induction of oxidative stress or their role in anxiety, cognitive impairment, and hypertension. Using our animal model of oxidative stress, we investigated the effects of a standardized freeze-dried powder made from fresh grapes, rich in grape-specific polyphenols, on the concurrence of hypertension-anxiety and memory impairment in rats. Furthermore, we examined the interesting yet complex role of extracellular signal-regulated protein kinase-1/2 (ERK-1/2), calcium/calcmodulin-dependent protein kinase type IV (CAMK-IV), cAMP response element–binding protein (CREB), and brain-derived neurotrophic factor (BDNF) as critical factors that potentially confer grape-induced neuroprotection.

Materials and Methods

Freeze-dried grape powder

Freeze-dried grape powder was provided by the California Table Grape Commission. The composition of the grape powder includes fresh red, green, and blue-black California grapes (seeded and seedless varieties), which were frozen, ground with food-quality dry ice, freeze-dried and re-ground, processed, and stored to preserve the integrity of the biologically active compounds. The powder contains resveratrol, flavans (including catechin), flavonols (including quercetin), anthocyanins, and simple phenolics (Supplemental Table 1). The grape powder was provided in small, sealed sachets. Upon receipt, the powder was stored at −80°C. The grape powder was dissolved in tap water at a concentration of 15 g/L. The powder was made fresh every day for feeding the rats. This dose was carefully chosen after conducting some pilot dose-response experiments. The chosen dose showed the most pronounced effects on rat behavior.

Animal model of oxidative stress

All experiments were conducted in accordance with NIH guidelines using approved protocols from the University of Houston Animal Care Committee. Male Sprague-Dawley rats (200–250 g) were acclimatized for 1 wk before any treatment and provided with rat chow and drinking water ad libitum. Picolab Rodent Diet 20 (catalog no. 5056) was purchased from LabDiet, Inc. and contains a mixture of 20% crude protein, 4.5% crude fat, 6% fiber, and 7% minerals. BSO (300 mg/kg body weight) was administered i.p. once daily for 7 d (7).

Four groups of rats were used: 1) control [C; injected with vehicle (7) and provided with tap water], 2) grape powder–treated (GP; injected with vehicle and provided with 15 g/L grape powder dissolved in tap water for 3 wk), 3) BSO-treated [injected with BSO (300 mg/kg body weight), i.p. for 7 d and provided with tap water], and 4) BSO plus grape powder–treated (GP+BSO; injected with BSO and provided with grape powder–treated tap water). The GP+BSO group was pretreated with grape powder for 2 wk before the 7-d BSO treatment commenced and continued to receive grape powder–treated water during BSO treatment. Body weight (223 ± 3.30 g), food intake (21.0 ± 3.20 g/d per rat), and fluid intake (21.0 ± 3.90 ml/d per rat) did not differ among the 4 groups. The 2 GP groups consumed 330 ± 3.00 mg grape powder/d.

Twenty-four hours after the last day of BSO treatment, memory tests were conducted followed by anxiety-like behavior tests. After completion of these tests, rats were subjected to blood pressure measurement, at the completion of which they were killed by decapitation. Blood, urine, and tissues were collected, and indices of oxidative stress were measured as previously published by us (7,8,16).

Anxiety behavior tests

An open-field test was conducted by a light-dark test, as previously published by us (7,8,16).

Open-field test. Rats were placed in the center of an open field (60 × 40 cm) and left free to explore the arena for 15 min; their movement was quantified by using the Opto-Varimex Micro Activity Meter v2.00 system (Optomax; Columbus Instruments), as previously published by us (7,8,16). Total time spent in the center of the arena, number of rearings, and fecal boli were registered.

Light-dark exploration. The light-dark box consists of a light and a dark compartment separated with a single opening for passage from 1 compartment to the other, and total time spent in the lighted area was recorded, as previously published by us (7,8,16).

Learning and memory-function tests

The radial arm water maze apparatus consisted of a black circular water pool containing 6 swim paths as described in our previous publication (17). The rats were randomly released at an arm different from the goal arm and allowed to swim and locate the platform. The rats were allowed 1 min for each learning trial or memory test. An error was counted when the rat entered more than halfway into an arm other than the goal arm or if the rat entered more than half of the goal arm but failed to approach the platform. The number of errors ranged from 1 to 7, because the rat could only swim into 7 arms within 1 min. If the rat failed to locate the platform within 1 min, it was manually guided to the platform and was scored with 7 errors. Upon reaching the platform, the rat was allowed 15 s of rest before the next trial. The rats were subjected to the first set of 6 learning trials (trials 1–6) followed by a 5-min rest and then another set of 6 learning trials (trials 7–12) and tested for short-term memory 30 min after the end of 12th trial. The rats were subjected to learning trials (trials 1–12) as above. At the end of the 12th trial, the rats were returned to their home cages and 24 h later subjected to a long-term memory test.

Blood pressure measurement

Blood pressure was measured as previously described (28). Briefly, rats were anesthetized with Inactin (Sigma-Aldrich) (100 mg/kg i.p.). Tracheostomy was performed to facilitate breathing. To measure blood pressure and to collect blood samples, the left carotid artery was catheterized with PE-50 tubing (ThermoFisher Scientific). This tubing was connected to a pressure transducer, which was connected to an amplifier (Grass LP122). Blood pressure was continuously recorded for 30 min by using the Grass PolyView Data Acquisition and Analysis Software systems (Astro-Med; Grass Instrument Division). After blood pressure measurement, aliquots of blood and urine were withdrawn, and plasma was isolated by centrifugation and kept frozen until further use.
Brain dissections and preparation of homogenates

Rats were anesthetized by using mild anesthesia (isoflurane, no. 57319–479–06; Phoenix Pharmaceuticals) immediately after behavior tests. The brains were quickly removed and rapidly frozen at \(-80^\circ\text{C}\) until analysis. The hippocampus, amygdala, and cortex were identified according to Paxinos and Watson (29) and grossly dissected out and homogenized; protein concentration was determined as previously published by us (16).

FIGURE 1 Analysis of markers of oxidative stress \(8\)-isoprostane and MDA in rats treated with vehicle, grape powder, \(\alpha\)-lipoic acid, \(\alpha\)-lipoic acid–treated (BSO), and grape powder plus BSO. The \(8\)-isoprostane concentrations in urine (A) and serum (B) were measured by using an Enzyme Immuno Assay kit (Cayman). (C) MDA was measured as previously described by us (7,16) and quantified by using the molar extinction coefficient \(1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1}\). (A, B) Means without a common letter differ, \(P < 0.05\). (C) Means without a common letter differ within each brain region, \(P < 0.05\). Bars represent means ± SEMs, \(n = 6–10\) rats/group. BSO, BSO-treated rats; C, vehicle-treated control rats; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats; MDA, malondialdehyde.

FIGURE 2 Examination of anxiety-like behavior tests including light-dark (A) and open-field (B–D) tests in rats treated with vehicle, grape powder, \(\alpha\)-lipoic acid–treated (BSO), and grape powder plus BSO. The open-field test determined center time (B), rearing (C), and fecal boli (D). Means without a common letter differ, \(P < 0.05\). Bars represent means ± SEMs, \(n = 10\) rats/group. BSO, BSO-treated rats; C, vehicle-treated control rats; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats.
different from those in the C group. Similarly, serum 8-isoprostane in the GP group was not significantly different from the C group. Malondialdehyde concentrations (marker of lipid peroxidation) were also significantly greater in the BSO group in the hippocampus (Fig. 1C), amygdala (Fig. 1D), and cortex (Fig. 1C) compared with the C or GP groups. Malondialdehyde concentrations were comparable between C, GP, and GP+BSO groups across the 3 brain regions.

**Anxiety-like behavior tests.** Light-dark and open-field tests were conducted to examine anxiety-like behavior. In the light-dark test, the rat is exposed to a novel environment with protected (dark compartment) and unprotected (light compartment) areas. An unwillingness to explore the lighted, unprotected area and more willingness to spend time in the dark during a 5-min test session is indicative of high-anxiety-like behavior. BSO rats spent significantly less time in the light compartment compared with C, GP, and GP+BSO rats (Fig. 2A). In the open-field test, BSO rats spent significantly less time in the center of the open field compared with C, GP, and GP+BSO rats (Fig. 2B). Rats that spend significantly more time exploring the unprotected center exhibit anxiolytic-like baseline behavior. Rearing recorded by the Optomax software in the open-field test indicated that BSO rats displayed significantly fewer rearings compared with the C, GP, and GP+BSO rats (Fig. 2C). Rearing is a typical rodent tendency and is demonstrative of their curious behavior. Reduced rearing is indicative of increased anxiety. Fecal boli are considered to be an activity-independent measure of anxiety-like behavior and were counted for each group at the end of the 10-min period of data collection during the open-field test. Fecal boli counts suggested that BSO rats had significantly greater numbers of fecal boli compared with C, GP, or GP+BSO rats (Fig. 2D).

**Learning and memory impairment.** BSO treatment significantly increased the number of errors made in the short-term memory test as compared to C, GP, and GP+BSO rats (Fig. 3A). Similarly, in the long-term memory test, BSO treatment significantly increased the number of errors compared with rats in the C, GP, and GP+BSO groups (Fig. 3B). GP rats exhibited similar learning and short- and long-term memory performance equivalent to control rats, indicating that grape powder treatment alone had no effect on long-term learning or short-term memory performance compared with control rats (Fig. 3A, B). Thus, under our experimental conditions, although grape powder did

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**Results**

**Indices of oxidative stress.** Urinary and serum 8-isoprostane concentrations were significantly greater in the BSO group compared with the C or GP groups (Fig. 1A, B). Mean urinary 8-isoprostane concentrations in the GP group were not significantly different from those in the C group. Similarly, serum 8-isoprostane in the GP group was not significantly different from the C group. Malondialdehyde concentrations (marker of lipid peroxidation) were also significantly greater in the BSO group in the hippocampus (Fig. 1C), amygdala (Fig. 1D), and cortex (Fig. 1C) compared with the C or GP groups. Malondialdehyde concentrations were comparable between C, GP, and GP+BSO groups across the 3 brain regions.

**Blood pressure measurement in rats treated with vehicle, grape powder, l-buthionine-(S,R)-sulfoximine-treated (BSO), and grape powder plus BSO.** Means (in left and right panels) without a common letter differ, *P* < 0.05. Bars represent means ± SEMs, *n* = 8–10 rats/group. BSO, BSO-treated rats; C, vehicle-treated control rats; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats.
not significantly affect learning and memory in normal rats, it prevented BSO-induced cognitive impairment.

Blood pressure measurement. Systolic and diastolic blood pressure of BSO rats showed a significant increase compared with that of C and GP rats, whereas GP+BSO rats showed blood pressure recordings closer to those of controls and a 20% decrease in blood pressure compared with BSO-alone rats (Fig. 4).

Assessment of ERK-1/2, GLO-1, GSR-1, CAMK-IV, CREB, and BDNF protein expression. Induction of oxidative stress via BSO treatment caused ERK-1/2 activation (phospho ERK-1/2 normalized to total ERK-1/2 protein) in the hippocampus, cortex, and the amygdala (Fig. 5). Moreover, ERK-1/2 activation in BSO-treated rats was significantly greater than in the control groups (C or GP) in the amygdala, hippocampus, and cortex. Grape powder treatment attenuated BSO-induced ERK-1/2 activation in the amygdala and the cortex but not in the hippocampus in GP+BSO rats (Fig. 5).

Furthermore, levels of GLO-1 (Fig. 6A), GSR-1 (Fig. 6B), CAMK-IV (Fig. 7A), phosphorylated CREB (P-CREB) (Fig. 7B), and BDNF (Fig. 7C) in the amygdala, hippocampus, and cortex were significantly less in BSO rats compared with control (C or GP) rats. Interestingly, grape powder treatment attenuated BSO-induced reduction in levels of GLO-1 (Fig. 6A), GSR-1 (Fig. 6B), CAMK-IV (Fig. 7A), P-CREB (Fig. 7B), and BDNF (Fig. 7C) in GP+BSO rats.

Discussion

The present study demonstrates that consumption of freeze-dried grape powder has a protective effect on anxiety-like behavior, memory function, and high blood pressure in rats, which could be attributed to the antioxidant capacity of the grape powder polyphenols (25).

Earlier, beneficial effects of grapes or resveratrol, a purified bioactive component of grapes, were reported on cognition, anxiety (25–27), and hypertension (30) in separate studies. None of the studies investigated their simultaneous effect on these 3 conditions, nor has anyone studied the effect of grapes on brain and behavior after induction of oxidative stress. Our study is the first to our knowledge to report the protective effect of grape powder on oxidative stress–induced anxiety-like behavior, memory loss, and hypertension in the same group of rats. Previously, we reported a protective role of the antioxidant tempol in BSO-induced anxiety-like behavior (7). Thus, 2 separate studies using 2 different antioxidant agents, tempol and grape powder, both suggest a protective role of antioxidants in anxiety-like behavior.

FIGURE 5 Analysis of ERK-1/2 activation in the hippocampus, amygdala, and cortex of rats treated with vehicle, grape powder, i-buthionine-(S,R)-sulfoximine (BSO), and grape powder plus BSO. ERK-1/2 activity was determined as previously published (16) (details described in the Methods section). Representative blots for phospho-ERK-1/2 (upper panel) and total ERK-1/2 (lower panel). Density ratios of phospho-ERK-1/2 and total ERK-1/2 are represented as bars. Means without a common letter differ within the hippocampus, amygdala, and cortex, P < 0.05. Bars represent means ± SEMs, n = 6–8 rats/group. BSO, BSO-treated rats; C/CON, vehicle-treated control rats; ERK-1/2, extracellular signal-regulated kinase-1/2; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats.

FIGURE 6 Examination of GLO-1 and GSR-1 protein levels in the hippocampus, amygdala, and cortex of rats treated with vehicle, grape powder, i-buthionine-(S,R)-sulfoximine (BSO), and grape powder plus BSO. Protein levels of GLO-1 (A) and GSR-1 (B) were determined by Western blotting. The upper panels shown in (A) and (B) are representative blots for GLO-1 (A), GSR-1 (B), and the protein loading control β-actin (A, B). Bar graphs in (A) and (B) are ratios of GLO-1 to β-actin and GSR-1 to β-actin, respectively. Means without a common letter differ within the hippocampus, amygdala, and cortex, P < 0.05. Bars represent means ± SEMs, n = 6–8 rats/group. BSO, BSO-treated rats; C/CON, vehicle-treated control rats; GLO-1, glyoxalase-1; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats; GSR-1, glutathione reductase-1.
Interestingly, BSO also caused short- and long-term memory impairment in rats, which was prevented with grape powder. Blood pressure was also analyzed in the same rats. Furthermore, consistent with previous reports (7,13–15), BSO rats had significantly higher blood pressure than control rats and the BSO-induced increase in blood pressure was prevented with grape powder treatment. These are important observations considering the high level of comorbidity reported between anxiety, hypertension, and learning-memory impairment (1–6).

Oxidative stress, which is an imbalance between cellular production of reactive oxygen species and the counteracting antioxidant mechanisms (31), may be the underlying cause of this comorbidity. Our molecular data have revealed some interesting clues to support this concept. We observed that the expression of 2 antioxidant enzymes involved in the oxidative stress pathway and implicated in anxiety-like behaviors (7,16,32), GLO-1 and GSR-1, were reduced in the hippocampus, amygdala, and the cortex of BSO-treated rats, which was prevented with grape powder treatment. It is possible that the reduced levels of GLO-1 and GSR-1 contributed to the failing antioxidant defense, which led to the anxiety-like behavior in rats.

Moreover, oxidative stress is reported to regulate gene expression (33,34) via activating many transcription factors (35), thus increasing the activity of many protein kinases while inactivating protein phosphatases (36). Pertinent to this, we found increased expression of the mitogen-activated protein kinase ERK-1/2 in the amygdala, cortex, and the hippocampus upon BSO treatment, which was prevented with grape powder treatment in the amygdala and the cortex but not in the hippocampus. This is most likely due to the fact that the protective effect of grape powder cannot supersede oxidative stress–induced, pronounced ERK-1/2 activation (+130%) observed in this region. ERK-1/2 has been previously reported to be upregulated upon induction of oxidative stress (36,37), and the role of ERK-1/2 in anxiety, stress, memory, plasticity, and depression (38–41) is also known. Our observations of increased ERK-1/2 activation upon induction of oxidative stress and prevention of ERK1/2 activation with grape powder are particularly interesting considering the previous report in which resveratrol was reported to reduce ERK-1/2 activation and thereby suppress expression of proinflammatory molecules, interleukin (IL)-1β and tumor necrosis factor (TNF)-α (42). Our results also fit well with our own observations in which BSO and xanthine/xanthine oxidase both increased proinflammatory cytokines and caused hypertension as well as anxiety-like behavior in rats (32). It is likely that grape powder attenuates ERK-1/2–mediated increase in inflammatory markers and in this manner confers neuroprotection.

The hippocampus was the most susceptible to oxidative stress–induced biochemical changes in ERK-1/2, GLO-1, GSR-1, CAMK-IV, P-CREB, and BDNF, followed by the amygdala and the cortex. This region is likely to be important for modulation via nutritional intervention. Exactly why these regional differences exist and the implications with regard to comorbidity remain to be determined. Some insights can be drawn from previous evidence. The involvement of the amygdala, cortex, and hippocampus in anxiety disorders (43,44), central control of blood pressure (45,46), and cognition (47) is known. Exactly which brain region is less or more critical than the others in regulation

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**FIGURE 7** Examination of CAMK-IV, CREB, and BDNF protein levels in the hippocampus, amygdala, and cortex of rats treated with vehicle, grape powder, l-buthionine-(S,R)-sulfoximine (BSO), and grape powder plus BSO. Protein expression levels of CAMK-IV (A), CREB [phospho (P)/total] (B), and BDNF (C) in the hippocampus, amygdala, and cortex were determined by using Western blotting. Upper panels are representative blots for CAMK-IV/β-actin (A), phospho-CREB/total CREB (B), and BDNF/β-actin (C). Bar graphs are ratios of CAMK-IV to β-actin, phospho to total CREB, and BDNF to β-actin, respectively. Means without a common letter differ within the hippocampus, amygdala, and cortex, P < 0.05. Bars represent means ± SEMs, n = 6–8 rats/group.

BDNF, brain-derived neurotrophic factor; BSO, BSO-treated rats; CON, vehicle-treated control rats; CAMKIV, calcium/calmodulin-dependent protein kinase type IV; CREB, cAMP response element–binding protein; GP, grape powder–treated rats; GP+BSO, grape powder plus BSO–treated rats; P-CREB, phosphorylated cAMP response element–binding protein.
of this comorbidity and which brain area is most susceptible to oxidative stress mechanisms and consequently amenable to a nutritional intervention is difficult to comment on. Perhaps an interconnection within these brain regions modulates oxidative stress, ultimately affecting comorbidity. For example, γ amino butyric acid projections transmit anxiety-related information from the amygdala to various centers in the brainstem (48,49) and from there primary noradrenergic projections connect to the hippocampus and cortex, creating an axis that determines the presence/absence or severity of this comorbidity.

Furthermore, we observed reduced protein expression of CAMK-IV, P-CREB, and BDNF in response to induction of oxidative stress, which was prevented with grape powder treatment. CAMK-IV is known to regulate CREB levels, which in turn reportedly modulate BDNF expression (50,51). Moreover, activation of CREB is considered important for learning and memory (52), whereas the role of BDNF in neuroprotection is well established (53). In fact, BDNF is downregulated during stress in rodent hippocampus, whereas antidepressant treatment prevents stress-induced decreased BDNF levels. Therefore, the inhibitory effect of oxidative stress on the phosphorylation of CREB, which eventually leads to depletion in BDNF, may contribute to learning and memory impairment as has been previously suggested for conditions associated with oxidative stress such as aging and Alzheimer disease (54). Although we have identified molecular targets that are amenable to a nutritional intervention, a limitation of this study is that it is not possible to pinpoint particular component(s) of grapes that potentially act in concert or in parallel with some or all of these pathways. In conclusion, it is likely that multiple signaling pathways involving antioxidant (7,16), antiinflammatory (32), and/or antiapoptotic (55) mechanisms enable the neuroprotective effect of grapes.

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Literature Cited