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Chronic purple grape juice consumption induces age-dependent changes on cognitive function in elderly women

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Abstract

Objective: To verify the chronic consumption of grape juice effects on weight, body mass index (BMI), cognitive function and the peripheral levels of global histone H4 acetylation, BDNF and inflammatory markers in elderly women according to age. Methods: This is a quasi-experimental study. The blood samples were collected before and after 30 days of grape juice supplementation (400 ml/daily) in women aged > 70 and < 70 years old. Weight, BMI and cognitive function, were measured before and after supplementation. Results: Grape juice consumption promoted a reduction on weight and BMI. This consumption also contributed to improve a cognitive function. These effects were more pronounced in the >70 years group. A tendency towards increased BDNF levels was observed in the >70 years group after intervention. Inflammatory markers and global histone H4 levels did not change. Conclusion: The chronic grape juice consumption is an interesting choice to promote health benefits in elderly women, which seem to act in an age-dependent manner.

Introduction

The current world scenario is characterized by a rapid and considerable increase in the elderly population [1]. This is critical information since the aging process is characterized by the progressive decline of physiological function that contributes to cognitive retardation and may increase the incidence of neurodegenerative diseases [2,3]. Thus, it is essential to elucidate the mechanisms involved during the aging process and investigate new approaches that can improve brain function. Recent evidences pointed out that both normal aging process and the physiopathology of neurodegenerative disease incidence and progression are linked to epigenetic pathways imbalance [4-6]. Epigenetic mechanisms modulate gene expression patterns without affecting the primary deoxyribonucleic acid (DNA) sequence in response to external factors and environmental cues such as diet and exercise [4,7,8].

Histone acetylation is an important epigenetic marker linked to enhanced transcriptional activity [9,10], catalyzed by histone acetyltransferases (HAT) [11]. Inversely, histone deacetylation, induced by histone deacetylases (HDAC) activity, is typically associated with transcriptional repression [12]. Interestingly, the HAT/HDAC imbalance appears to be pivotal in triggering the neurodegenerative cascade of events [6]. In this sense, increased levels of HDAC activity was detected in the hippocampi from aged rats [7]. Congruently, lower levels of global histone H4 acetylation in hippocampi from aged rats [7]. These findings could be related to the decreased levels and/or expression of genes that exert pivotal role on memory and synaptic plasticity during the aging process, including the Brain Derived Neurotrophic Factor (BDNF) [13]. In fact, lower levels of peripheral BDNF are associated to the cognitive deficits in elderly people [14].

It is also reported that the aging process is accompanied by remodeling changes in the immune system which altered cytokine profile represents one of the most dramatic disturbances [15]. The imbalance between pro- and anti-inflammatory cytokines were observed, including an elevation of pro inflammatory cytokins such as tumoral necrosis factor alpha (TNF-α), interleukin-6 (IL-6 and interferon-gamma (INF-γ) [16,17]. This status seems to be responsible for a chronic pro inflammatory profile, named inflammaging [18], recently described an interaction between the imbalance on immune system and cognitive deficits in humans. Furthermore, experimental data also reported that the aging-related memory decline is linked to histone H4 hypoacetylation levels and the modulation of inflammatory parameters [5].

Dietary components may be able to control cytokine deregulation in many diseases, in particular, natural bioactive compounds contribute to reduce the inflammation [19,20]. In this context, polyphenol compounds found in grape juice have been associated with multiple health benefits including improvement in cognition and neuronal function with aging [21-23]. Experimental studies conducted by our research group demonstrated the neuroprotective effects of chronic purple grape juice consumption in several brain areas from rats submitted to different damage models [24-26].

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However, the molecular mechanisms through which purple grape juice consumption improves cognitive outcomes during the aging process are not yet elucidated. Therefore, our aim was to evaluate the effect of chronic purple grape juice consumption according to the age on cognitive performance, peripheral levels of global histone H4 acetylation of BDNF, inflammatory profile and anthropometric parameters in healthy elderly women.

Methods

Grape juice

Grape juice used was the integral grape juice provided by Suvalan® brand, packaged at 200ml, all from the same batch (1771-13). Throughout the tests, we observed the expiration dates of the juice, phenolic compounds and chemical analysis. Table 1 shows the principal characteristics of the grape juice used. The content of total polyphenols was 53.6±0.18 mg catechin/ml. The epicatechin and naringin appeared in higher concentrations. The physical and chemical parameters of the grape juice used attend to the Brazilian legislation.

Subjects

Sample size was set according to other studies performed in humans and with the aid of Winpepi program, considering 30% coefficient of variation, 90% power and a 0.05 significance level [27-30].

The authors invited the elderly women through posters in hospitals and social networks. Women under sixty years old, carriers of neurodegenerative disease, diabetes (both types), and smokers were excluded from the study. Thirty-seven elderly women over sixty years old were interested in participating in the research, but there were two withdrawls and thirty-five completed the study (n = 35). The desistance occurred in the first week of research. The first abandoned for experiencing accentuated epigastric pain and linked this to grape juice consumption; the second needed to undergo a surgery.

The volunteers were instructed to keep the same routine regarding diet, exercise, and medication during the thirty days of research to prevent possible confounders. During the intervention period, one month, the researchers each week certified that the volunteers consumed 400 ml/day of grape juice. All the experimental procedure was performed at the same period.

Experimental design

This was a quasi-experimental study, aimed to evaluate interventions but that do not use randomization. Similar to randomized trials, quasi-experiments aim to demonstrate causality between an intervention and an outcome [31,32]. Considering the difficulty of obtaining a placebo group or a control group (without any grape derivate consumption) we justify our choice in doing a quasi-experimental design without control groups. This difficulty could be explained because our state is one of the most important regions in grape cultivation, and for this reason we have a high consumption of these products.

The survey was conducted in three meetings. At the first one the search mechanism and the criteria for blood collection were explained; the other two meetings were divided into before and after supplementation, following according to Figure 1. In these two meetings, anthropometric measurements were collected and Mini Mental State Examination (MMSE) was applied to evaluate the cognitive performance. Blood samples (10ml) were collected for the measurement of global histone H4 and BDNF levels as well inflammatory parameters.

The volunteers received all the required amount of juice for the thirty days of research in the before meeting, consuming 400ml daily. E-mails, text messages, and phone calls were made in order to remind volunteers about the consumption requirements.

Evaluation of anthropometric parameters

These parameters were analyzed before and after supplementation. Semi-analytical Balance Welmy with coupled stadiometer (Santa Barbara D'Oeste, São Paulo, Brazil) was used to evaluate the height (m) and weight (kg). Waist circumference (cm) and hip circumference (cm) were measured with a flexible tape. Body mass index (BMI= kg/m^2) was obtained using the formula weight/height^2. The investigators responsible for these measures were the same, before and after the intervention.

Cognitive performance assessment

Cognitive performance was measured using the Portuguese version of the Mini Mental State Examination (MMSE) [33]. The test provides a brief evaluation of the cognitive domains including orientation, registration, attention, recall, language, and constructional praxis. Patients' scores range from 0 to 30, with low scores indicating greater cognitive impairment. Cutoffs were used to grade severity of change as mild, moderate, or severe. Namely, mild cognitive impairment was considered when the patient was rated 20–24 points, moderate cognitive impairment with 16–19 points, and severe cognitive impairment with

Table 1. Grape Juice Characteristics.

Analysis	Result	Method
Total Phenolic Compound (mg catechin /mL)	53.6 ± 0.18	Ribèrau-Gayon, 2003
trans-Resveratrol (mg/L)	0.79 ± 0.01	MCMurtrey et al., 1994
Catechin (mg/L)	1.03 ± 0.03	HPLC
Epicatechin (mg/L)	5.38 ± 0.06	HPLC
Naringin (mg/L)	6.96 ± 0.21	HPLC
Quercetin (mg/L)	0.59 ± 0.06	HPLC
Total Acidity (g % tartaricacid)	0.54 ± 0.00	IN nº24 08/09/2005 (In, 2005)
Volatile acidity (g/100g)	0.00	IN nº24 08/09/2005 (In, 2005)
º Brix	14.28 ± 0.00	IN nº24 08/09/2005 (In, 2005)
Relative density on 20ºC	1.058 ± 0.00	IN nº24 08/09/2005 (In, 2005)
Organoleptic Characteristics	Normal	IN nº24 08/09/2005 (In, 2005)
pH	3.33 ± 0.01	IN nº24 08/09/2005 (In, 2005)
Alcoholic grade (%v/v)	0.00	IN nº24 08/09/2005 (In, 2005)
Relation ºBrix/Total acidity (g % tartaric acid)	26.44 ± 0.00	IN nº24 08/09/2005 (In, 2005)
Data presented as mean±standard deviation. HPLC - High Performance Liquid Chromatography.		

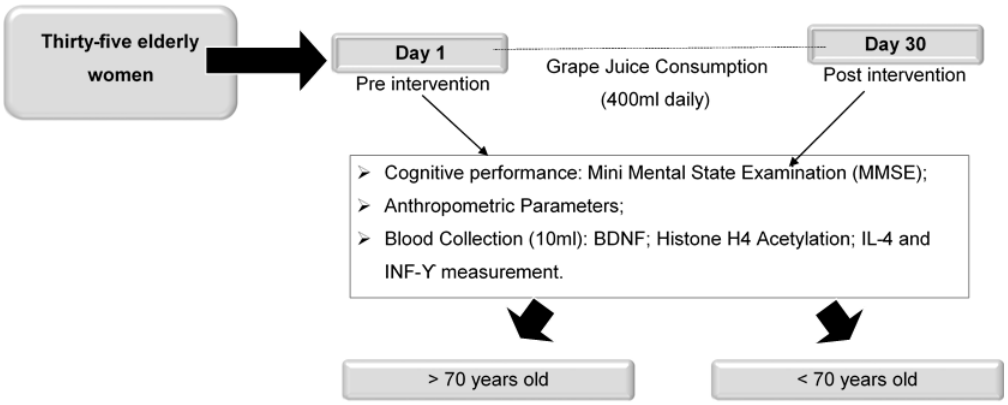

Figure 1. Flowchart of experimental design in the initial and final meetings. Only in the initial meeting. MMSE – Mini Mental State Examination.

15 or fewer points. The cutoff score for dementia is 19 points. The test sensitivity was 87%, and specificity was 82%.

Extraction of peripheral blood mononuclear cells (PBMC)

PBMC were extracted by Ficol gradient. Histopaque® (Sigma-Aldrich 1077) was added in the collected sample in a 1:1 proportion in a conical tube and then centrifugation was held at 1500 rpm for 30 minutes at room temperature. After that, the "buffer coat" was removed from the portion between plasma and Histopaque®. The "buffer coat" was washed 5 times with phosphate buffered saline solution (PBS – pH 7.4) and then centrifugation was held at 1800 rpm for 10 minutes at room temperature. The formed pellet was collected and used for evaluation of acetylation levels of histone H4. Cell viability was always higher than 95%. The remaining plasma was collected and stored in conical tubes (1.5 ml) at -80°C for later determination of BDNF levels.

Determination of BDNF levels

BDNF levels were determined with the enzyme-linked immunosorbent assay (ELISA) method, from Sigma-Aldrich commercial kit (catalog number RAB0026) according to manufacturer's instructions. To perform the test the remaining plasma samples from the leukocyte extract were used. These, together with BDNF specific standards, were added to ELISA microplate and incubated for 2.5 hours at room temperature.

Subsequently, the solutions were discarded and the same plate was washed 4 times with wash buffer (PBS, Tween 20 0.01%). After washing, the secondary antibody bound to biotin was added and incubated for 1 hour at room temperature with gentle agitation. The plate was again washed with wash buffer and streptavidin solution was added. The plate was incubated at room temperature for 45 minutes with gentle agitation. The solution was discarded and the plate passed through the washing process. Tetramethylbenzidine (TMB) was added, and incubated for 30 minutes at room temperature under light deprivation with gentle agitation. The stop solution was added and the plate was read in a spectrophotometer at a wavelength of 450 nm.

Determination of global histone H4 acetylation levels

The global histone H4 acetylation levels were determined using the Global Histone H4 Acetylation Assay Kit (Colorimetric Detection, catalog number P-4009, EpiQuik USA) according to the manufacturer's instructions. The extracted leukocyte cells from the blood samples were processed with a specific nuclear extract lysis-buffer followed by histone extraction. The extraction was carried out with trichloroacetic acid, chloridric acid and acetone; the formed pellet was used for the H4 acetylation detection. The samples were incubated with the capture antibody followed by incubation with detection antibody. After, the samples were incubated with developing solution followed by the addition of the Stop Solution. The absorbance was measured on a spectrophotometer at a wavelength of 450nm. The global histone H4 acetylation levels were expressed as ng/mg protein. Protein content was performed using the Biuret method. For this, we used the commercial kit of Total Protein (Labtest Diagnostics S/A, Lagoa Santa, MG, Brazil), following the manufacturer's methodology.

Determination of cytokine levels

The systemic levels of INF-y and IL-4 were determined by ELISA with specific kits (Mini ELISA Development Kit, 900-M21, PeproTech Inc., NJ) and following the manufacturer's recommendations. To avoid errors due to intra-assay sensitivity, all cytokine's analyses were performed on the same plate on the same day. Plates of 96 wells were incubated overnight with capture antibody, anti-INF-y or IL-4 diluted in 1PBS buffer. After blocking for 1 h to avoid nonspecific binding, 100 ml of standard INF-y or IL-4 and serum samples were placed. The cytokines were detected by horseradish peroxidase-labeled monoclonal antibody to each target after the addition of 100 µl anti-human INF-y or IL-4 biotinylated antibodies, they were placed in each well and incubated for 2 h at room temperature. The microplate was washed to remove unbound enzyme-labeled antibodies. The amount of horseradish peroxidase bound to each well was determined by the addition of 100 ml substrate solution. The reaction was stopped by the addition of 100 ml of 1M sulfuric acid, and the plates read at 450 nm (ThermoPlate, São Paulo, Brazil). The concentrations of cytokines were determined by interpolation from standard curve and presented as pg/ml.

Ethical procedures

All participants read and signed the Informed Consent (IC), according to Resolution 466/12 of the Brazilian National Health Council. The Research Ethics Committee of the Centro Universitário Metodista - IPA approved the study under protocol 1.174.858.

Statistical analysis

Data were checked for normality by Shapiro-Wilk test. Parametric data presented as mean ± standard deviation and compared pre-post supplementation through paired t-test. For the variables that deviated from normality, we used the nonparametric Wilcoxon test (median and interquartile range). Comparison between groups was performed

unpaired Student t-test (for parametric variables) or Mann-Whitney U test (nonparametric variables). Correlations performed through Spearman test. All analyses were performed by SPSS 20.0 (IBM., NY, USA). All statistical tests were two-tailed and were performed using a significance level of $p<0.05$.

Results

Thirty five elderly women participated in the present study, 16 of them were in the <70 years old group and 19 were in the >70 years old group (Table 2).

Firstly, we evaluated the effect of chronic grape juice consumption on cognitive performance, anthropometric and biochemical parameters. Table 2 shows a significant reduction on body mass and BMI as well improved the cognitive performance ($p=0.001$) after supplementation in both groups (> 70 and <70 years old).

When the sample was separated in > 70 and <70 years old groups, it was observed a clear impact of age in these responses, as highlighted in Table 3. Specifically, the grape juice consumption did not alter body mass and BMI in the <70 years women, while diminished significantly both parameters in the oldest group. Regarding the cognitive performance, the improvement after the grape juice consumption appears in the <70 and >70 years old groups. Interestingly, Δ value in the >70 group was higher when compared to <70 women ($p=0.04$), as demonstrated in Figure 2. Finally, the >70 group showed a tendency towards increased BDNF levels ($p=0.069$) after the supplementation (Table 3).

However, neither age nor grape juice supplementation altered significantly the systemic cytokine and global histone H4 levels ($p>0.05$; Table 3).

Discussion

In the current study, we provide the first evidence reporting the beneficial effects of grape juice consumption on antropometric parameters and cognitive performance in elderly women. A novel and important finding that emerged from our study is that response and seem to vary in an age-dependent manner.

The improvement on cognition after supplementation was evaluated through the MMSE. This is a validated and effective instrument that asses five domains of cognition in elderly taken as orientation with regard to time and place, registration of words, attention and calculation, recall, and language [33,34].

In agreement to our findings, other studies conducted with elderly people also showed that the consumption of beverages rich in polyphenol such as blueberry juice improved the cognitive abilities [21]. The beneficial effect of grape juice on brain function was also highlighted in experimental models. Some authors demonstrated that treatment with grape polyphenol preparation prevented peripheral metabolic abnormality as well improved brain synaptic plasticity in a mouse, which is pivotal for the learning and the memory process [35]. In addition, other study with animal model for Alzheimer's disease suggests that grape juice consumption for 21 days is able to improve the learning and memory functions evaluated through the passive avoidance learning test [36]. Taken together, these findings support the idea that chronic purple grape juice consumption is a potential tool able

Table 2. Pre-post Comparison.

	Pre supplementation	After supplementation	Δ%	p
Age (years)	69.49 ± 6.97	-	-	-
Body mass (kg)	66.00 (16,325)	65.11 (17,775)	-0.37 ± 0.89	0.011
BMI (kg/m²)	26.354 (6.42)	26.298 (5.977)	-0.11 ± 0.40	0.03
INF-γ (pg/mL)	6.23 ± 1.43	6.26 ± 1.02	0.11 ± 1.35	0.69
IL-4 (pg/mL)	4.09 ± 1.67	4.58 ± 1.45	0.24 ± 1.46	0.66
Ratio IL-4/INF-γ	1.80 ± 0.98	1.49 ± 0.51	-0.17 ± 1.07	0.45
BDNF (pg/mL)	721.37 (310.88)	808.26 (308.09)	304.71 ± 932.48	0.12
Histone H4 acetylation levels (ng/mg of protein)	756.22 (498.40)	564.73 (477.54)	62.19 ± 3107.90	0.35

Data presented as mean ± SD (parametric values) or median with interquartile range (nonparametric values).Pre-after comparison through paired Student t-test or Wilcoxon test. BMI, Body mass index; INF-γ, Interferon-gamma; IL-4, interleukin-4; BDNF, Brain-derived neurotrophic factor.

Table 3. Pre-post comparison with age.

		Pre	After	P	Δ%	p≠
Body Mass (Kg)	<70	66.92 ± 12.71	66.66 ± 12.56	0.254	-0.30 ± 0.77	0.912
	>70	66.40 (15.99)	65.60 (17.325)	0.015	-0.35 ± 1.01	
BMI (Kg/m2)	<70	27.69 ± 5.971	27.62 ± 5.909	0.553	-0.13 ± 0.35	0.816
	>70	26.35 (6.154)	25.712 (6.139)	0.035	-0.09 ± 0.45	
INF-ϒ (pg/mL)	<70	6.13 ± 1.43	6.01 ± 1.53	0.164	-0.35 ± 0.77	0.117
	>70	5.61 ± 1.5	6.16 ± 0.94	0.280	0.54 ± 1.64	
IL-4 (pg/mL)	<70	4.06 ± 1.47	4.14 ± 1.43	0.860	0.27 ± 1.54	0.928
	>70	4.26 ± 1.54	4.30 ± 1.56	0.667	0.21 ± 1.46	
Ratio IL-4/INF-ϒ	<70	1.72 (0.94)	1.41 (0.95)	0.286	-0.07 (1.69)	0.519
	>70	1.18 (0.8)	1.25 (0.42)	0.859	0.02 (0.93)	
BDNFlevels (pg/ml)	<70	817.23 (459.46)	883.42 (300.81)	0.717	119.7 (703.52)	0.196
	>70	600.26 (275.26)	741.63 (446.51)	0.069	215.40 (412.29)	
Histone H4 Acetylation levels (ng/mg of protein)	<70	634.12 (849.92)	709.52 (716.36)	0.438	-166.78 (438.18)	0.297
	>70	816.90 (482.2)	469.41 (386.43)	0.586	-346.26 (1006.08)	

Data presented as mean ± SD (parametric values) or median with interquartile range (nonparametric values). Pre-after comparison through paired Student t-test or Wilcoxon test. p≠ Inter-group comparison by Unpaired Student t-test or Mann Whitney U-Test. BMI, Body mass index; INF-γ, Interferon-gamma; IL-4, interleukin-4; BDNF, Brain-derived neurotrophic factor.

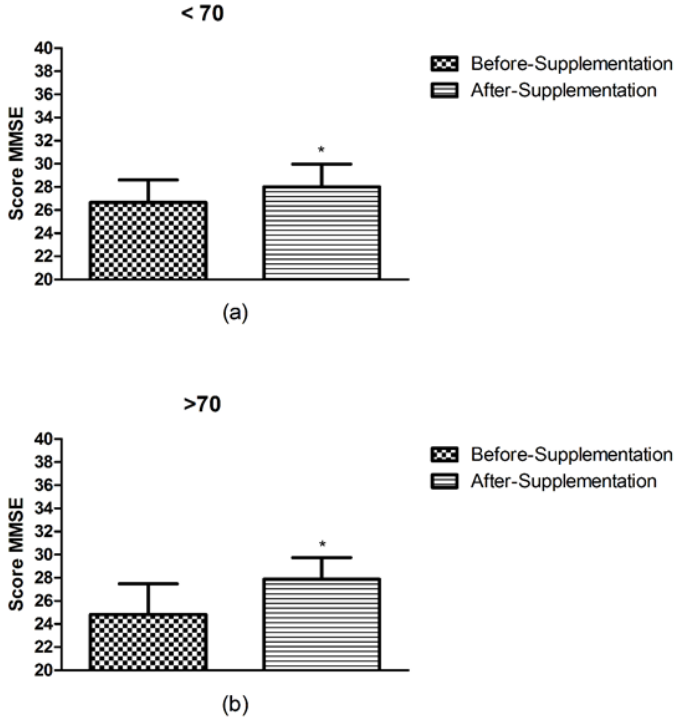

Figure 2. The effect of chronic grape juice consumption on MMSE test. (a) age group under seventy years old. (b) age group above seventy years old. Data presented as mean ± SD (parametric values). Before and after comparison through paired Student t-test. * Statistic difference after supplementation ($p<0.005$).

to improve cognitive performance not only during the physiological age process as well in neurodegenerative conditions [37].

A remarkable point to discuss is that the cognitive performance improvement seem to be more evident in the aged women, since the >70 group showed a greater difference in MMSE scores compared to the <70 women after the supplementation. This data might be related to the increased tendency on BDNF levels in the >70 group since this neurotrophin exert an important role in both learning and memory abilities [38].

Emerging evidences have shown that being overweight is negatively associated with cognitive functions, whereas weight loss may improve it in middle-aged and elderly people [39-41]. In support of this view, a recent study showed that diet modification promoted weight loss in overweight postmenopausal women and increased hippocampal activity during memory encoding. The authors also state that weight loss may decrease risk of developing dementia [42]. Corroborating this idea, another study demonstrated that long-term consumption of a high-calorie diet could inhibit autophagy function and facilitate neuronal loss in the hippocampus, which in turn aggravate age related cognition impairment [43]. In accordance, besides the improvement of cognitive function, the present study also found a significant reduction on body mass and BMI after grape juice consumption in all individuals. Besides, these changes were more significant in the > 70 years old women.

Consistent with these considerations, we postulate that the enhancement on cognitive ability following grape juice supplementation in elderly women is related, at least in part, to the body mass reduction. It is also plausible to suppose that the positive effects of this intervention are more pronounced with advancing age.

Although substantial evidences have been supporting the pivotal role of epigenetic regulation on polyphenol compounds mechanisms of action [44-46], the chronic grape juice consumption was not able to do it. Importantly, the cited studies are focused in the context of the polyphenol diet use on the management and therapeutic targets of age-related diseases, differently from our sample, consisted of healthy elderly women. Then, we might hypothesize that polyphenol compounds could act as the source of epigenetic modifications preferentially in pathological condition during the aging process.

Finally, it is important to consider that the intervention used in our study was chronic. On the other hand, experimental and clinical studies already reported that the impact of external stimuli such as exercise on acetylation levels status could be dynamic and with short-lived and acute outcomes, without any delayed effect [4,47,48]. This response may be act as a protective mechanism to maintain the homeostasis of the transcriptional machinery. Further studies should investigate the acute effect of grape juice consumption to elucidate this question.

Conclusion

Summarizing, our data indicate that chronic grape juice consumption is able to contribute in cognitive performance ameliorate in elderly women, which seems to be related, at least in part, to BMI and body mass reduction. This beneficial response might vary through lifespan, been more evident in the oldest group, and did not engage the modulation of inflammatory and epigenetic signals. This pioneer study opens new avenues for further investigations in order to elucidate the molecular mechanism involved behind this phenomenon.

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Declaration of conflicting interests

The Authors declare that there is no conflict of interest.

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