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Comparing fermented food and vitamin E on exercise-mediated Nrf2/ARE activation in middle-aged/ elderly: A randomized, double-blind clinical study

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ABSTRACT

Background: There is a compelling need to emphasize the importance of deepening the rationale behind functional foods research in molecular biology. Indeed, establishing stringent criteria to evaluate functional food interventions in disease prevention and as adjunctive therapeutic strategies offers significant opportunities for health promotion.

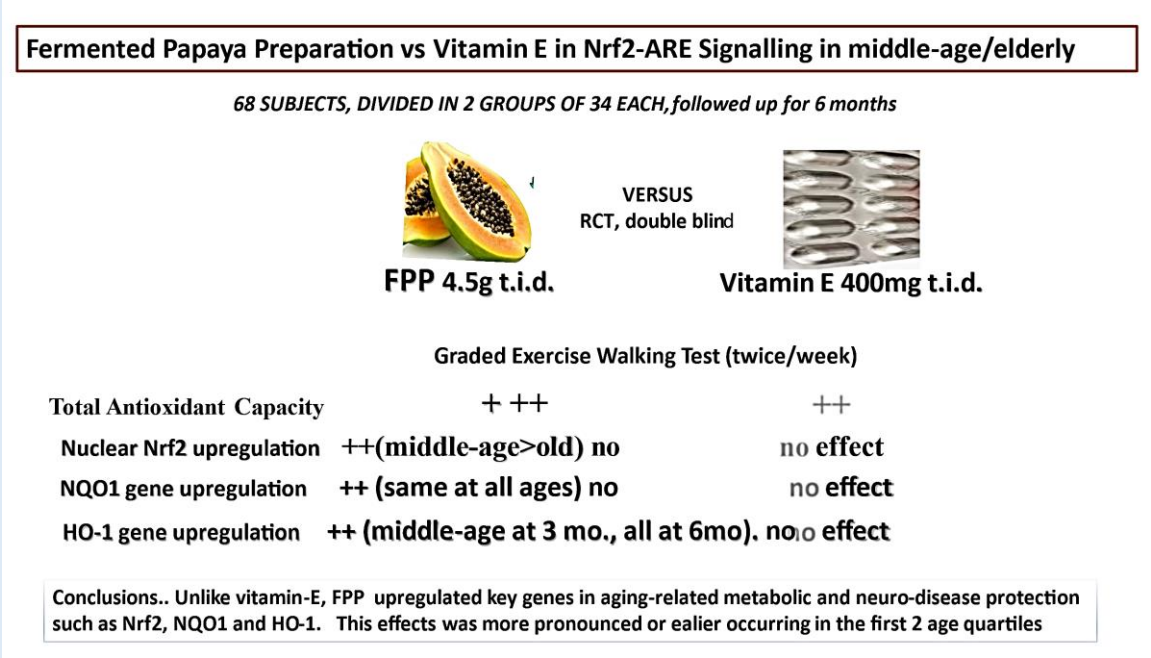
Objective: This study investigated the effects of fermented papaya preparation (FPP®) and vitamin E on gene expression in middle-aged/elderly individuals, leveraging FPP's established antioxidant and immune-regulatory benefits.

Methods: Graded Exercise Walking Test was administered twice weekly for 6 months. Total Antioxidant capacity (TAC) and gene expression (Nrf2, NQO1, HO-1) in PBMC were measured monthly.

Results: Throughout the 6-month study, routine biochemistry and BMI remained stable. Both treatments significantly improved Total Antioxidant Capacity (TAC), Vitamin E exhibiting earlier effects (3 months). Notably, FPP® supplementation led to sustained over-expression of nuclear Nrf2 in all subjects, surpassing Vitamin E's effects. NQO1 gene expression was rapidly and consistently upregulated in the FPP group, exceeding baseline and Vitamin E levels throughout the study. HO-1 gene expression was upregulated by FPP at 1 month in younger age quartiles (Q1-2) and at 3 and 6 months in all

subjects, regardless of age and gender. In contrast Vitamin E did not significantly impact Nrf2, NQO1, or HO-1 gene expression.

Conclusion: These findings demonstrate that FPP, unlike Vitamin E, potentiates Nrf2 signaling, triggering a downstream epigenetic cascade. Notably, our clinical data reveal that physical exercise combined with functional food supplementation partially restores impaired Nrf2 signaling in elderly individuals.



INTRODUCTION

Disrupted redox homeostasis conceptually links several aging hypotheses, such as oxidative stress and the impaired molecular signaling response theory [1-3], although some controversies exist regarding the best monitoring tool [4]. Our body is equipped with a system aimed to stimulate antioxidant gene expression, as shown by in vitro and in vivo Keap1- Nuclear factor erythroid2-related factor2 (Nrf2) testing [5-7].

This ubiquitously present factor holds a key position in cellular protection against the aforementioned deleterious phenomena by upregulating redox epigenetics and scavenging carriers. This action is also associated with cytoprotection, phase II detoxifying enzymatic arrays, and chaperone proteins [8-11].

Recent studies in Nrf2 knock-out mice suggest this factor may exert a potential antiatherogenic and cardioprotective effect [12-14]. Redox abnormalities following Nrf2 system failure have been implicated in neurodegenerative diseases [15-18]. However, functional food interventions using phytohormones have been limited only to in vitro or in vivo experiments [19-21], and complex longevity inferences at the cellular level [22]. Conversely, a dysfunctional Nrf2 system and its blunted antioxidant response are linked to the aging process [23], as oxidative stress-related aging phenomena have been reported in clinical studies, sparking extensive research into functional foods [24-27]. Notably, promising clinical data have been published [28-30].

Despite this, the fundamental goals of functional health foods remain a cornerstone [31-32] and may help to unveil the unsuspected effects of natural extracts [33]. Functional genomic studies have deepened our understanding of personalized approaches to health. Recent investigations have shown that Nrf2/ARE system activity varies depending on single nucleotide

polymorphisms in the promoter site [34]. These findings are relevant when considering the interrelationships between the Nrf2-ARE system and SARS-CoV-2 outcome [35] and environmental pesticide mutagenicity [36-37]. The importance of proper functional food analysis and its integration into evidence-based medicine has recently been emphasized [38-40]. This is crucial for developing synergistic health benefit strategies [38-46].

Thus, the present study aimed to test the Nrf2 gene expression and related functional genomics cascade in middle-aged/elderly individuals treated with vitamin E or a patented fermented papaya preparation (FPP®, Osato Research Institute, Gifu, Japan, Patent Number: 6401792), which has a consistent scientific literature supporting its benefits. Specifically, FPP has demonstrated significant redox and immune system nutrigenomic benefits through key molecular signaling pathways [47-48]. Our study population consisted of healthy middle-aged and elderly individuals (43-75 years old) with no or minor diseases, focusing on Nrf2 gene expression for NADPH dehydrogenase-quinone-1 (NQO1) and Heme-oxygenase 1 (HO-1).

MATERIALS AND METHODS

The study design adhered to the Declaration of Helsinki and its subsequent amendments and was approved by the ReGenera Research Association committee on October 12, 2023. This prospective, randomized clinical trial compared a commercial antioxidant in a double-blind manner.

Potential participants who agreed via email or phone to participate and met the pre-screening inclusion and exclusion criteria (Table 1), were invited for a visit.

Table 1. Pre-screening inclusion and exclusion criteria

Inclusion/accepted criteria	Exclusion criteria
Participants were required to be untrained individuals with an active lifestyle, either working or retired, and have a positive socializing attitude. Additional, criteria included normal blood pressure, being a non-smoker or having quit smoking at least six months prior, maintaining a stable diet and sleep pattern, and not taking food supplements or having stopped supplementation at least three months before entering the study. Furthermore, eligible participants had to be free form musculoskeletal problems and be teetotalers or infrequent light drinkers, with no consumption of spirits.	Participants were excluded from the study if they had a history of or were currently experiencing any of the following conditions: cancer, stroke, myocardial infarction, diabetes, thromboembolism, hypertension, obesity, or gastrointestinal diseases such as ulcers, ulcerative colitis, or Crohn's disease. Additional exclusion criteria included malabsorption syndromes, hematological or endocrinological diseases, chronic fatigue syndrome, post-COVID-19 complications, suspected post-COVID-19 vaccination side effects, fibromyalgia, rheumatological or autoimmune diseases, or depression and other major mental health disorders. Furthermore, individuals who had undergone surgery within the past six months (excluding minor skin procedures), used food supplements within the past three months, smoked or quit smoking less than six months prior, engaged in weekly intensive physical exercise, consumed heavy amounts of alcohol, used illicit drugs, had unstable dietary habits, experienced sleep disturbances, or regularly consumed high-caffeine beverages were also excluded.

All subjects provided written informed consent and underwent a comprehensive evaluation, including medical history, physical examination, laboratory tests, and heart rate variability (HRV) analysis. Anthropometric measurements and resting blood pressure were assessed concurrently with resting EKG. All participants were within 20% of their ideal body weight.

Body mass index (BMI) was calculated: Heart Rate Variability (HRV) was assessed using the Body Health Analyzer Pro (Binacor, Poulsbo, Washington, USA) system,

a Bluetooth-equipped finger device connected to specialized software. This software analyzes heart rhythm variation and characteristics to infer various related sympathetic and parasympathetic variables. Before each test, participants rested for 10 minutes in a quiet room. Raw data were visually analyzed to detect potential movement artifacts, ectopic beats, or abnormal breathing patterns.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice guidelines conducted the trial. A dietary analysis confirmed that the average nutrient intake aligned with the Mediterranean diet requirements (15% protein, 40-45% carbohydrates, and 35-38% fat, comprising 22-24% monounsaturated fatty acids (MUFA), 8-12% extra-virgin olive oil, 8-10% saturated fatty acids (SFA), and 4% polyunsaturated fatty acids (PUFA)). Participants meeting all inclusion criteria were randomized in a 1:1 ratio using a random number generator to receive either a 3g sachet of FPP® plus a filmed white tablet of cellulose twice daily or a 3g sachet of sugar plus a filmed white tablet of Vitamin E (400 IU) twice daily.

Graded Exercise Walking Test (GEWT): The Graded Exercise Walking Test (GEWT) was designed to be subject-friendly, focusing on exercise stress rather than performance enhancement [49]. This approach mimics real-life situations, incorporating aerobic and anaerobic components. The GEWT protocol, applied on the 1st and 3rd day of each week, involved a 6min of warm-up (from 4.5k/h to 6.5km/h), 7-12min of intensive aerobic test (from 7k/h to 8k/h) and 12-22min of anaerobic phase (from 8k/h to exhaustion or 11km/h). Blood tests were conducted the day before GEWT protocol initiation, within 2 hours after GEWT completion, and the following morning. This schedule was repeated weekly for 6 weeks.

Blood samples (10 mL) were collected by venipuncture at baseline, 1-month, 3-month, and 6-month visits, using anticoagulant EDTA tubes. After dispersion and centrifugation (2500 rpm, 15 minutes, 25 °C), plasma aliquots were transferred and stored at -80 °C until laboratory analysis. Routine biochemistry was performed using an automatic biochemistry analyzer (Beckman Coulter). Antioxidant intake from individual diets was estimated by linking NHANES 24-hour dietary recall data to an antioxidant and Total Antioxidant Capacity (TAC) database for commonly consumed foods.

TAC Assessment: Plasma samples were analyzed for lipid-soluble antioxidant capacity using the following protocol. A 200 µL plasma sample was mixed with 400 µL ethanol. Then, 800 µL hexane was added, and the mixture was briefly shaken and centrifuged at 1000× g for 5 minutes. The lipidic phase (200 µL) was collected, dried under nitrogen flow, and stored at -80 °C until further analysis. For analysis, the dried extract was dissolved in 200 µL methanol and centrifuged at 5000× g for 1 minute. The resulting supernatant (120 µL) underwent Photochem analysis, utilizing Trolox-assisted calibration and a photosensitizer. This sample was then used for the final TAC calculation as follows:

TAC = [Quantity (nmol) × Dilution × Trolox Molarity (ng/nmol)]/pipetted volume (µL)

Nrf2 Genotype Study: DNA was isolated from whole-blood samples using a modified phenol-chloroform extraction method. The -653A/G, -651G/A, and -617C/A genotypes were determined by direct sequencing of the -738 to -461 region within the NFE2L2 promoter. This approach identified three single nucleotide polymorphisms (SNPs) in the encoding gene, specifically -653A/G, -651G/A, and -617C/A. The polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 8 minutes. The sequencing reactions were analyzed on a Perkin Elmer ABI 3100 DNA Sequencer (Applied Biosystems, California, USA).

PBMNc gene studies: Whole blood was collected and diluted 1:1 with phosphate-buffered saline (PBS). The diluted blood was then layered onto a Ficoll-Histopaque gradient (Sigma-Aldrich) and centrifuged at 800×g for 25 minutes at 20°C. Following centrifugation, the lymphocyte-rich layer was retrieved and washed twice with phosphatase inhibitor-supplemented PBS (ThermoFisher, Waltham, USA) at 400×g for 10 minutes at 4°C. The supernatant was discarded, and the cell pellet was washed with a buffer solution. Cell counting was performed using an automated system (BioRad CD375, Hercules, USA). Subsequently, 2×10^6 PBMC cells were lysed and suspended in 100 µL of RNA lysis buffer (Ambion Biotech, Austin, USA), and stored at -80°C until further analysis. Additionally, aliquots of 1×10^6 and 3×10^6 cells/mL were fractionated for whole cell and nuclear fractions, respectively, for western blot assay.

A rapid, efficient, and practical method for subcellular fractionation was employed [40]. Briefly, 3 × 10^6 cells were lysed in a solution containing phosphatase and protease inhibitors in 0.1% Triton PBS, gently mixed, and microcentrifuged. The resulting supernatant (cytosolic fraction) was separated and mixed with 4x Laemmli sample buffer in a 3:1 ratio. The pellet (nuclear fraction, approximately 20 µL) was resuspended in 1000 µL of 0.1% Triton PBS solution, followed by a 10-second centrifugation step. After discarding the supernatant, the nuclear fraction was resuspended in Laemmli buffer. For whole-cell lysates, 1 × 10^6 cells were transferred to microcentrifuge tubes and lysed using 0.1% Triton PBS with protease-phosphatase inhibitor mixture, then mixed with Laemmli buffer. Nuclear fractions and DNA-rich whole-cell lysates were sonicated on ice at level 2 for 5 seconds, three times. All samples were heated at 100°C for 5 minutes and immediately stored at -80°C until analysis.

Western Blot Analysis: Following SDS-PAGE-assisted electrophoretic separation, samples were transferred to nitrocellulose membranes. Nuclear fractions were incubated with a rabbit polyclonal primary antibody against Nrf2 (ThermoFisher, PA-kit). The samples were then co-incubated with anti-mouse monoclonal and anti-rabbit secondary antibodies. Band intensity was analyzed using ImageJ Version 1.56 (National Institutes of Health, Bethesda, MD, USA) by comparing the optical density of each band to baseline values using a digital image analyzer. The results were expressed as a ratio in arbitrary units.

Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed using the Qiagen RNeasy Plus Mini Kit (Qiagen, Gaithersburg, USA) to analyze RNA at baseline and after 2 and 6 months. RNA quality and quantity were assessed using a Thermo Nanodrop (ThermoFisher), and integrity was verified by agarose gel electrophoresis. Next, samples were denatured at 65°C for 5 minutes, followed by RNA-cDNA conversion using reverse transcription reagents (BioRad, Hercules, USA). A DNase step was included to remove residual genomic DNA. For RT-qPCR, primer pairs specific to NQO1 and HO1 were used, with Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) as an internal control gene. Reactions were triplicated on a CFX96-Touch (Bio-Rad, Hercules, USA). mRNA levels were normalized to the housekeeping gene GAPDH. The gene-specific primer pairs are outlined in Table 2.

Table 2. Forward and reverse primers for RT – qPCR

Primers	Forward 5’ to 3’	Reverse 5’ to 3’
Nrf2	TCAA A T CCATGTCCTGCT	ACTCTCGCCCAAGCGAGA
NQO1	GGATTGGACCGAGCTGGAA	AATTGCAGTGAAGATGAAGGCAAC
HO-1	AAGAGGCCAAGACTGCGTTC	GGTGTCATGGGTCAGCAGC
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Statistical analysis: The data are presented as median/interquartile range (IQR). The Hardy-Weinberg equilibrium, comparisons of allele frequencies between different databases, and exclusion rates by sex and age were analyzed using the χ2 test. Comparisons of mean values between different genotype groups were analyzed using the Kruskal-Wallis test, followed by post hoc analysis with the Holm method. Statistical significance was defined as a p-value less than 0.05. All statistical analyses were performed using R software, version 3.6.2.

RESULTS

Compliance with both treatments was highly satisfactory. Isolated, minor protocol deviations were deemed to have no significant impact on the study outcomes, and therefore, no subjects were excluded. These deviations included one subject in Group A and two subjects in Group B; one subject in Group A who engaged in 3 hours of intensive cycling during the week, but fully rested for the subsequent three days; one subject in Group B who took a non-steroidal anti-inflammatory drug (NSAID) for two days to alleviate back pain after prolonged driving. Notably, no subject reported any digestive or allergic symptoms throughout the study.

Heart Rate Variability (HRV) and Root Mean Square of the Successive Differences (RMSSD) were evaluated. No significant differences were observed between the two treatment groups throughout the 6-month study period. Notably, the oldest age quartile showed a non-significant trend towards a higher stress index and reduced vagal balance at baseline. However, unlike the complete normalization observed at 3 and 6 months in the FPP-treated group, this trend persisted in the vitamin E-supplemented group, although the difference did not reach statistical significance.

Routine biochemistry and body mass index (BMI) remained unchanged from baseline to the end of the study (data not shown). A non-significant trend of decreased BMI and total cholesterol was observed in both groups.

Total Antioxidant Capacity (TAC): Both treatments resulted in a significant increase in TAC plasma levels. Specifically, the vitamin E group showed increased TAC levels starting from the 1st month of observation, while the FPP-treated group demonstrated sustained increases throughout the 6-month study, irrespective of gender and age quartiles (Table 3).

Table 3. Effect of FPP® and Vitamin E on TAC plasma values along 6 months of treatment

TAC (mmol/L)	Baseline	1 month	3 months	6 months	p-value
FPP®	829.1 ± 94.2	821.1 ± 86.2	842.7 ± 102.4	911.2 ± 93.7 *	<0.01
Vitamin E	837.6 ± 88.9	829.3 ± 95.4	918.4 ± 97.8 *	928.2 ± 77.8 *	<0.01

Time Course Modification of Total Antioxidant Capacity (TAC) during Treatment with FPP® or Vitamin E: Both treatments yielded a statistically significant improvement in TAC levels. However, the Vitamin E effect was observed earlier, after only 3 months of treatment. Baseline gene analysis revealed that 38 subjects (55.8%) had the C/C genotype, 28 (41.2%) had the C/A genotype, and 2 (2.9%) had the A/A genotype. Sequencing of the Nrf2 gene demonstrated that, compared to the Vitamin E-supplemented group, the FPP-treated group exhibited overall significant overexpression of nuclear Nrf2 in all subjects ($p < 0.01$ vs. Vitamin E, Fig. 1). Furthermore, when analyzing the data by age group, the oldest age quartile showed statistically improved Nrf2 gene expression ($p < 0.05$ vs. baseline and $p < 0.01$ vs. Vitamin E). However, this age group displayed significantly less pronounced FPP-induced Nrf2 gene transcription than the first two quartiles ($p < 0.05$, Fig. 1).

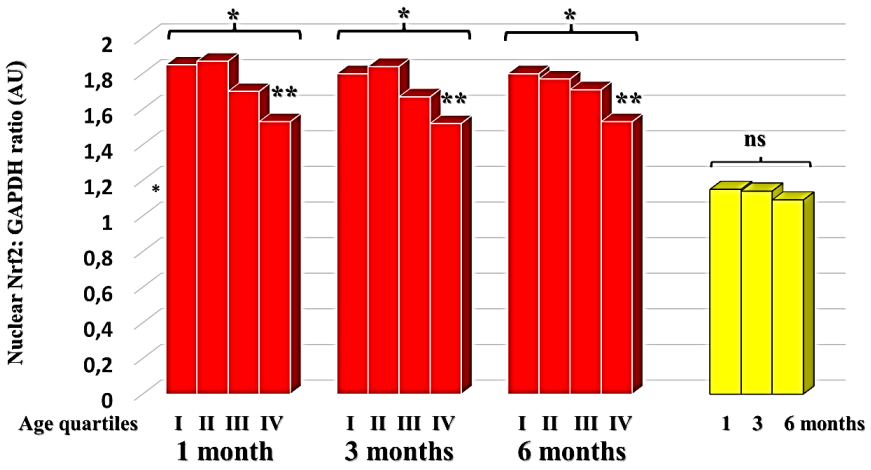


Figure 1. Effect of FPP and vitamin E on time-course variation of Nrf2 gene expression.

Gene expression responses are normalized to each subject's baseline value, set at 1, to facilitate comparison of differences in expression levels. The results are presented as follows: red bars, FPP-treated group; yellow bar, vitamin E-treated group. Statistical significance is indicated as: *p < 0.01 vs. baseline and vitamin E group; **p < 0.05 vs. the first two age quartiles; ns, not significant. Notably, these data remained unchanged when stratified by the two main Nrf2 genotypes. However, the limited data from the A/A genotype precluded meaningful analysis. Additionally, gender did not influence the results. NQO1 gene expression was significantly upregulated in the FPP-treated group as early as the 1st-month observation (p < 0.01 vs. baseline and vitamin E intervention, Fig. 2). This increase persisted throughout the 6-month study period (p < 0.01 vs. baseline and vitamin E group, Fig. 2). Neither age nor gender had a significant impact on these findings.

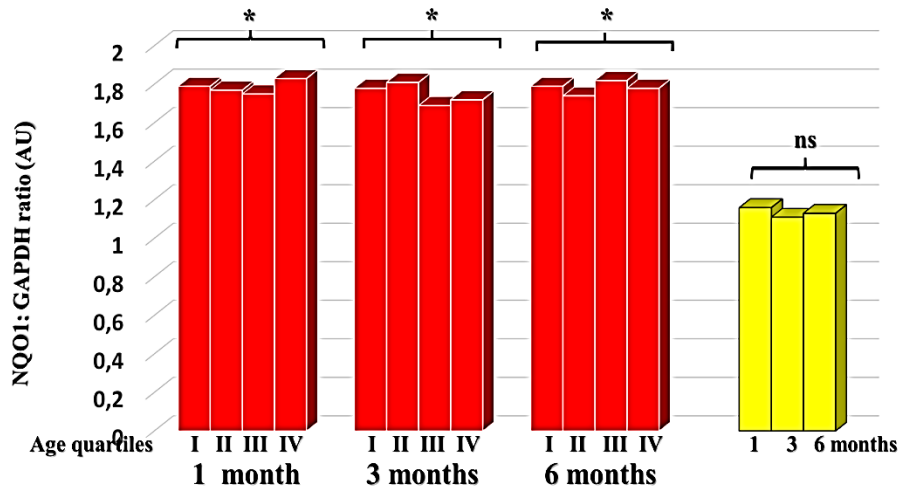


Figure 2. Effect of FPP and vitamin E on time-course variation of NQO1 gene expression.

Data are normalized to each subject's baseline value, set at 1, to facilitate comparison of possible differences in absolute expression levels. Similar patterns were observed for HO-1 gene expression. Notably, at the 1st-month observation, the beneficial effects of FPP® were limited to the first two younger age quartiles (p < 0.05 vs. baseline and vitamin E, Fig. 3). However, by the 3rd and 6th months of observation, all FPP-treated subjects, regardless of age and gender, showed significant upregulation of HO-1 gene expression (p < 0.05 vs. baseline and vitamin E, Fig. 3).

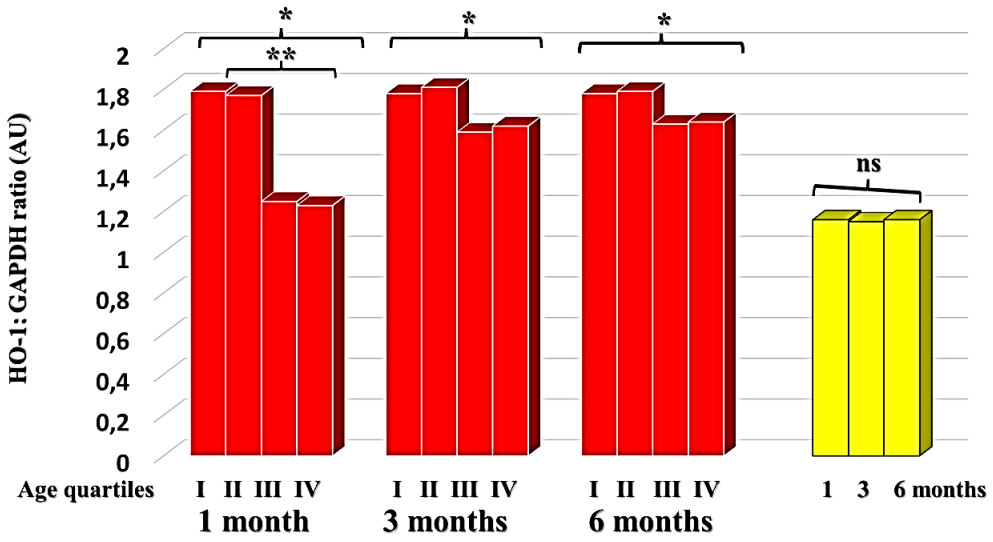


Figure 3. Effect of FPP and vitamin E on time-course variation of HO-1 GENE expression.

Data are adjusted to each subject's baseline value, set at 1, to facilitate comparison of possible differences in absolute expression levels. This normalized baseline value serves as a reference point for evaluating changes in expression levels. The results are presented as follows: red bars, FPP-treated group; yellow bar, vitamin E-treated group. Statistical significance is indicated as: *p < 0.01 vs. baseline and vitamin E group; **p < 0.05 vs. the first two age quartiles; ns, not significant. Notably, HO-1 mRNA response was unaffected by gender or other factors.

Furthermore, analysis of heart rate variability (HRV) revealed that the root mean square of successive differences (RMSSD), a measure of sympathetic/parasympathetic balance, was significantly lower in the older age quartiles (last two quartiles) compared to the younger middle-aged groups (first two quartiles, p < 0.01, Table 4). Although not statistically significant, a trend towards increased RMSSD was observed in the FPP-treated group at 3 and 6 months.

Table 4. HRV variability assessment: time course and effect of supplementations

FPP®	Baseline	1 month	3 months	6 months
First 2 age quartiles *	67 ± 8	62 ± 5	71 ± 9	70 ± 4
Last 2 age quartiles	47 ± 4	48 ± 6	51 ± 8	53 ± 5
VITAMIN E				
First 2 age quartiles *	63± 4	58 ± 6	67 ± 2	69 ± 3
Last 2 age quartiles	45 ± 9	39 ± 8	46 ± 4	48 ± 5

*p<0.05 vs second two age quartiles.

CONCLUSION

The present study demonstrated that vitamin E exhibited a strong beneficial antioxidant capacity, as evidenced by Total Antioxidant Capacity (TAC) assessment. Notably, this effect appeared to occur earlier than the functional food examined, FPP. However, our study has limitations. We did not measure vitamin E status in our participants, and our redox balance analysis was not comprehensive. Therefore, we cannot rule out the possibility that our findings, particularly in the elderly, may have been influenced by vitamin E status [50-52]. Regarding vitamin E's poor effectiveness on Nrf2 signaling, age-related

gastritis, which may be asymptomatic in elderly subjects, could potentially have a negative impact [53]. Nevertheless, subsequent studies suggest that gastric pH does not significantly influence vitamin E metabolism [54]. Only in cases of age-related atrophic gastritis accompanied by significant pancreatic insufficiency could malabsorption of fat-soluble vitamins occur [55]. However, our subjects' minimal alcohol consumption made chronic pancreatitis unlikely. Interestingly, supplementation with RRR-alpha-tocopherol failed to enhance physical activity-related ex-vivo induction of nitric oxide and HO-1 gene expression [56].

Oxidative stress is known to alter the cysteine structure of Keap1, thereby inhibiting Nrf2 degradation and facilitating the increase of nuclear mRNA transcripts [57-61]. Consequently, attention has focused on Nrf2-modifying interventions using antioxidants, such as resveratrol and lipoic acid, explored in experimental models of age-related diseases [62-63]. Our study in relatively healthy subjects demonstrated that a nutraceutical intervention with FPP enhanced Nrf2 gene expression. However, elderly subjects (defined as the last age quartile, 67-75 years old) exhibited a less pronounced response compared to middle-aged subjects (43-59 years old, $p < 0.05$). This finding aligns with evidence suggesting that antioxidant responses decline with advancing age [64-66]. Nonetheless, FPP induced positive Nrf2 upregulation, and combining it with regular exercise may synergistically overcome defective antioxidant responses [67-69]. Notably, our analysis revealed that Nrf2 genotype did not influence the response. The A/A genotype (Nrf2 rSNP-617), associated with increased risk of clinical disease, such as hypertension in never-smoking healthy subjects [70, 71], was rare in our population (2 subjects, 2.9%). In contrast, FPP supplementation induced robust and stable NQO1 mRNA upregulation at 3 and 6 months, regardless of age. This finding is particularly relevant given that Nrf2 and NQO1 expression decline with aging [72], compromising the antioxidant defense system's efficiency [73-74]. Moreover, HO-1 and NQO1 dysfunction have been implicated in the pathogenesis of central nervous system diseases [75-76].

Consistent with the established knowledge of aging-associated reductions in cardiac vagal modulation [77], our study found that elderly subjects exhibited lower heart rate variability (HRV). While prolonged and regular physical exercise may potentially improve this parameter, it was beyond the scope of our study. Interestingly, a recent experimental study in diabetic rats demonstrated that allopurinol, a synthetic antioxidant, enhanced Nrf2 and HO-1 gene expression, thereby significantly improving diabetic cardiomyopathy-related autonomic nervous system imbalance [78]. Given our present findings on FPP-induced upregulation of the Nrf2-ARE system, the potential benefits of nutraceutical intervention on HRV in healthy elderly subjects' merit further investigation in more extensive studies. Notably, Nrf2 deletion has been shown to markedly reduce the ability to modify HRV responses to environmental stressors [79]. Moreover, these parameters intersect with the concept that psychobiological resilience is a prominent factor in the epigenetics of aging [80]. Our previous study found that this nutraceutical significantly decreased oxidative stress and upregulated HO-1 in otherwise healthy, stressed individuals [81].

Although specific in vitro confirmation is lacking, these preliminary data suggest that FPP may potentially act as an Nrf2-ARE activator. This activation would disrupt basal ubiquitin-dependent degradation of Nrf2 by the 26S proteasome, thereby freeing nuclear Nrf2 to accumulate and trigger gene induction involved in cell cycle control, apoptosis, and antioxidant defense. Furthermore, Nrf2 activation may enhance mitochondrial function and adenosine triphosphate (ATP) production. Our study focused on the synergistic effects of moderately intensive walking exercise and a specific functional food, demonstrating effective activation and sustained engagement of the Nrf2-ARE system. Unlike

high doses of vitamin E, this combination offers significant health benefits by protecting a diverse range of target proteins from proteasomal degradation. Notably, this nutrigenomic effect partially restored cellular redox homeostasis, even in older individuals, who typically exhibit weakened responsiveness in the Keap1-Nrf2 signaling system, albeit to a minor extent. These findings align with previous research by Gounder et al. [82] and Baar et al. [83]. A health-driven dietary approach remains a cornerstone of health strategies, providing direct benefits for healthy individuals and collateral advantages for those with health complications [84]. Furthermore, rigorous studies on functional foods and their novel mechanisms strategically advance our understanding [85-90].

Conflict of interest statement: The authors declare that they have no known competing financial interests that could influence the work reported in this paper.

Abbreviations: PBMCs: Peripheral Blood Mononuclear Cells; FPP®: Fermented Papaya Preparation; BMI: Body Mass Index; HO-1 Heme oxygenase-1; NQO1 NAD(P)H dehydrogenase (quinone 1); Nrf2 - Nuclear factor erythroid 2-related factor 2; ARE - antioxidant response elements.

Contribution: J.C., A.L., and M.K. conceived and discussed the research plan. C.A., A.N., and R.R. oversaw the clinical aspects. F.H., C.A., and A.A. discussed and evaluated the nutritional aspects. J.C., A.M., and A.N. discussed and evaluated the biochemical aspects.

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REFERENCES

1. Castejon-Vega B, Cordero MD, Sanz A. How the Disruption of Mitochondrial Redox Signaling Contributes to Ageing. Antioxidants (Basel). 2023 Mar 29;12(4):831. DOI: https://doi.org/10.3390/antiox12040831
2. Jackson MJ. Exercise-induced adaptations to homeostasis of reactive oxygen species in skeletal muscle. Free Radic Biol Med. 2024 Oct 18; 225:494-500. DOI: https://doi.org/10.1016/j.freeradbiomed.2024.10.270.
3. Debès C, Papadakis A, Grönke S, Karalay Ö, Tain LS, Mizi A, et al. Aging-associated changes in transcriptional elongation influence longevity. Nature. 2023 Apr;616(7958):814-821. DOI: https://doi.org/10.1038/s41586-023-05922-y.
4. Luo J, Mills K, le Cessie S, Noordam R, van Heemst D. Ageing, age-related diseases and oxidative stress: What to do next? Ageing Res Rev. 2020 Jan;57:100982. DOI: https://doi.org/10.1016/j.arr.2019.100982.
5. Buffenstein R, Jarvis JU. The naked mole rat--a new record for the oldest living rodent. Sci Aging Knowledge Environ. 2002 May 29;2002(21):pe7. DOI: https://doi.org/10.1126/sageke.2002.21.pe7.
6. Almukainzi M, El-Masry TA, Selim H, Saleh A, El-Sheekh M,et al. New Insight on the Cytoprotective/Antioxidant Pathway Keap1/Nrf2/HO-1 Modulation by Ulva intestinalis Extract and Its Selenium Nanoparticles in Rats with Carrageenan-Induced Paw Edema. Mar Drugs. 2023 Aug 22;21(9):459. DOI: https://doi.org/10.3390/md21090459
7. Barreto R, Kawakita S, Tsuchiya J, Minelli E, Pavasuthipaisit K et al. Metal-induced oxidative damage in cultured hepatocytes and hepatic lysosomal fraction: beneficial effect of a curcumin/absinthium compound. Chin J Dig Dis. 2005;6(1):31-6. DOI: https://doi.org/10.1111/j.1443-9573.2005.00184.x
8. Baird L, Yamamoto M. The Molecular Mechanisms Regulating the KEAP1-NRF2 Pathway. Mol Cell Biol. 2020 Jun 15;40(13):e00099-20. DOI: https://doi.org/10.1128/MCB.00099-20.
9. Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim Biophys Acta Mol Cell Res. 2018 May;1865(5):721-733. DOI: https://doi.org/10.1016/j.bbamcr.2018.02.010.
10. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. Physiol Rev. 2018 Jul 1;98(3):1169-1203. DOI: https://doi.org/10.1152/physrev.00023.2017.
11. Murakami S, Kusano Y, Okazaki K, Akaike T, Motohashi H. NRF2 signalling in cytoprotection and metabolism. Br J Pharmacol. 2023 Sep 15. DOI: https://doi.org/10.1111/bph.16246.
12. Zhang Q, Liu J, Duan H, Li R, Peng W, Wu C. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the

protection of vascular endothelial cells from oxidative stress. J Adv Res. 2021 Jul 6;34:43-63.
DOI: https://doi.org/10.1016/j.jare.2021.06.023.

13. Chen QM, Maltagliati AJ. Nrf2 at the heart of oxidative stress and cardiac protection. Physiol Genomics. 2018 Feb 1;50(2):77-97. DOI: https://doi.org/10.1152/physiolgenomics.00041.2017.
14. Luo X, Weng X, Bao X, Bai X, Lv Y, Zhang S, Chen Y, Zhao C, Zeng M, Huang J, Xu B, Johnson TW, White SJ, Li J, Jia H, Yu B. A novel anti-atherosclerotic mechanism of quercetin: Competitive binding to KEAP1 via Arg483 to inhibit macrophage pyroptosis. Redox Biol. 2022 Nov; 57:102511. DOI: https://doi.org/10.1016/j.redox.2022.102511.
15. Chakkittukandiyil A, Sajini DV, Karuppaiah A, Selvaraj D. The principal molecular mechanisms behind the activation of Keap1/Nrf2/ARE pathway leading to neuroprotective action in Parkinson's disease. Neurochem Int. 2022 Jun; 156:105325. DOI: https://doi.org/10.1016/j.neuint.2022.105325
16. Uruno A, Yamamoto M. The KEAP1-NRF2 system and neurodegenerative diseases. Antioxid Redox Signal. 2023 Feb 3. DOI: https://doi.org/10.1089/ars.2023.0005
17. A A, W C, N N, L M, M D, Zhang DD. α-Syn overexpression, NRF2 suppression, and enhanced ferroptosis create a vicious cycle of neuronal loss in Parkinson's disease. Free Radic Biol Med. 2022 Nov 1; 192:130-140. DOI: https://doi.org/10.1016/j.freeradbiomed.2022.09.015.
18. Kim S, Indu Viswanath AN, Park JH, Lee HE, Park AY, et al. Nrf2 activator via interference of Nrf2-Keap1 interaction has antioxidant and anti-inflammatory properties in Parkinson's disease animal model. Neuropharmacology. 2020 May 1; 167:107989. Epub 2020 Feb 5. PMID: 32032607. DOI: https://doi.org/10.1016/j.neuropharm.2020.107989.
19. Rathore AS, Singh SS, Birla H, Zahra W, Keshri PK, et al. Curcumin Modulates p62-Keap1-Nrf2-Mediated Autophagy in Rotenone-Induced Parkinson's Disease Mouse Models. ACS Chem Neurosci. 2023 Mar 29. DOI: https://doi.org/10.1021/acschemneuro.2c0070
20. Wang Q, Botchway BOA, Zhang Y, Liu X. Ellagic acid activates the Keap1-Nrf2-ARE signaling pathway in improving Parkinson's disease: A review. Biomed Pharmacother. 2022 Dec; 156:113848. DOI: https://doi.org/10.1016/j.biopha.2022.113848.
21. Marotta F, Mao GS, Liu T, Chui DH, Lorenzetti A et al. P. Anti-inflammatory and neuroprotective effect of a phytoestrogen compound on rat microglia. Ann N Y Acad Sci. 2006 Nov; 1089:276-81. DOI: https://doi.org/10.1196/annals.1386.033
22. Morgunova, G.V., Klebanov, A.A,. Marotta, F. et al. Culture medium pH and stationary phase/chronological aging of different cells. Moscow University Biological Sciences Bulletin. 2017, 2, 47-51.
23. Yu C, Xiao JH. The Keap1-Nrf2 System: A Mediator between Oxidative Stress and Aging. Oxid Med Cell Longev. 2021 Apr 19; 2021:6635460. DOI: https://doi.org/10.1155/2021/6635460
24. Nicholas-Okpara Viola A.N., Adegboyega M, Oben J, et al Exploring the Potential of Bioactive Compounds as Interventions for Dementia: Current Insights and Future Directions. Functional Food Science, Vol. 4 No. 5: 166 – 179, May 2024. DOI: https://DOI.org/10.31989/ffs.v4i5.1329
25. Keita Takanashi K, Dan K, Shinozuka T et al. Functional ingredient derived from processed brown rice extract activates human macrophage and promotes its shifting from mitochondrial to glycolytic energy metabolic activation. Vol. 4 No. 4: 53: 153 - 165, April 2024 DOI: https://doi.org/10.31989/ffs.v4i4.1322
26. Martirosyan D, Ashoori R.M., Mikaeili S.A. et al. Inflammatory factors and immunoglobulins alterations in subjects with type 2 diabetes mellitus treated with squalene, Functional Food Science, 08, 30: 181-197, 2022. DOI: https://doi.org/10.31989/ffs.v2i8.979
27. Babalola, O.Y, Lawa I.O., Akinwumi I.A. et al., Comparative evaluation of phytochemical, in vitro antioxidant activities and elemental composition of the fruit, leaves, and stem bark of Tetrapleura tetraptera. Functional Food Science, Vol. 3 No. 12: 317-328, December 2023 DOI: https://doi.org/10.31989/ffs.v3i12.1273
28. Bertuccelli G, Zerbinati N, Marcellino M et al, Effect of a quality-controlled fermented nutraceutical on skin aging markers: An antioxidant-control, double-blind study. Exp Ther Med. 2016 Mar;11(3):909-916. DOI: https://doi.org/10.3892/etm.2016.3011.
29. Hossein Mirmiranpour H., Ashoori M.R., Mikaeili A.S., et al. The effect of squalene on lipid profile and some oxidative biomarkers in patients with type 2 diabetes mellitus. Functional Food Science, Vol. 2 No. 7: 144-156, July 2022. DOI: https://doi.org/10.31989/ffs.v2i7.949
30. Marotta F, Pavasuthipaisit K, Yoshida C, et al. Relationship between aging and susceptibility of erythrocytes to oxidative damage: in view of nutraceutical interventions. Rejuvenation Res. 2006 Summer;9(2):227-30. DOI: https://doi.org/10.1089/rej.2006.9.227.
31. Martirosyan, D., Alvarado, A. Functional Foods Regulation System: Proposed Regulatory Paradigm by Functional Food Center. Functional Food Science, Vol. 3 No. 11: 275-287, November 2023 DOI: https://doi.org/10.31989/ffs.v3i11.1265
32. Martirosyan, Lampert, D.T., Lee, M. A comprehensive review on the role of food bioactive compounds in functional food science. Functional Food Science 2022; 3(2): 64-78. DOI: https://doi.org/10.31989/ffs.v2i3.906
33. SamieeRad F, Sofiabadi M, Bahadoran E et al. Evaluation of propolis hepatotoxicity in male rats. Functional Food Science, Vol. 4 No. 5: 204 - 215 May 2024.

DOI: https://doi.org/10.31989/ffs.v4i5.1344

34. Boettler U, Volz N, Teller N et al. Induction of antioxidative Nrf2 gene transcription by coffee in humans: depending on genotype? Mol Biol Rep. 2012 Jun;39(6):7155-62. DOI: https://doi.org/10.1007/s11033-012-1547-6.
35. Marotta F, Chui DH, Jain S et al., Effect of a fermented nutraceutical on thioredoxin level and TNF-alpha signalling in cirrhotic patients. J Biol Regul Homeost Agents. 2011 Jan-Mar;25(1):37-45.
36. Marotta F, Koike K, Lorenzetti A, Jain S, Signorelli P et al. Regulating redox balance gene expression in healthy individuals by nutraceuticals: a pilot study. Rejuvenation Res. 2010 Apr-Jun;13(2-3):175-8. DOI: https://doi.org/10.1089/rej.2009.0950
37. Cuadrado A, Pajares M, Benito C. et al., Can Activation of NRF2 Be a Strategy against COVID-19? Trends Pharmacol Sci. 2020 Sep;41(9):598-610. DOI: https://doi.org/10.1016/j.tips.2020.07.003
38. Martirosyan, D, Lampert, D.T., Lee, M. A comprehensive review on the role of food bioactive compounds in functional food science. Functional Food Science 2022; 3(2): 64-78. DOI: https://doi.org/10.31989/ffs.v2i3.906
39. Adany, A. Kanya, H. Martirosyan, D. Japan's health food industry: An analysis of the efficacy of the FOSHU system. Bioactive Compounds in Health and Disease 2021; 4(4):63-78. DOI: https://www.doi.org/10.31989/bchd.v4i4.795
40. Chen L, Zhang T, Ge M, Liu Y, Xing Y, Liu L, Li F, Cheng L. The Nrf2-Keap1 pathway: A secret weapon against pesticide persecution in Drosophila Kc cells. Pestic Biochem Physiol. 2020 Mar; 164:47-57. DOI: https://doi.org/10.1016/j.pestbp.2019.12.008
41. Yaduvanshi SK, Srivastava N, Marotta F, et al. Evaluation of micronuclei induction capacity and mutagenicity of organochlorine and organophosphate pesticides. Drug Metab Lett. 2012 Sep 1;6(3):187-97. DOI: https://doi.org/10.2174/187231281120603000
42. Elgadir,M.A., Chigurupati, S., Mariod, A.A. Selected potential pharmaceutical and medical benefits of phenolic compounds: Recent advances. Functional Food Science 2023; 3(7): 108-128. DOI: https://doi.org/10.31989/ffs.v3i7.1118
43. Radwan, H., Ai Hitotsumatsu, I., Miyata, T. Identification of potent antioxidant bioactive peptides from the soluble proteins of chicken egg yolk. Functional Food Science 2023; 3(6)63-78. DOI: https://doi.org/10.31989/ffs.v3i6.1110
44. Cizmarova, B., Hubkova, B., Birkova, A. Quercetin as an effective antioxidant against superoxide radical. Functional Food Science 2023; 3(3): 15-25. DOI: https://doi.org/10.31989/ffs.v3i3.1076
45. Mirmiranpour, H., Reza Ashoori, M., Seyed Mikaeili, A., Pezeshki, P., Serani. A., Vassar, R., Martirosyan. The effect of squalene on lipid profile and some oxidative biomarkers in patients with type 2 diabetes mellitus. Functional Food Science 2022; 2(7): 144-156. DOI: https://doi.org/10.31989/ffs.v2i7.949
46. Vaiserman AM, Marotta F. Longevity-Promoting Pharmaceuticals: Is it a Time for Implementation? Trends Pharmacol Sci. 2016 May;37(5):331-333. DOI: https://doi.org/10.1016/j.tips.2016.02.003.
47. Marotta F, Safran P, Tajiri H, Princess G, Anzulovic H, et al. Improvement of hemorheological abnormalities in alcoholics by an oral antioxidant. Hepatogastroenterology. 2001 Mar-Apr;48(38):511-7. PMID: 11379344.
48. Marotta F, Chui DH, Jain S, Polimeni A, Koike K, Zhou L, Lorenzetti A, Shimizu H, Yang H. Effect of a fermented nutraceutical on thioredoxin level and TNF-alpha signalling in cirrhotic patients. J Biol Regul Homeost Agents. 2011 Jan-Mar;25(1):37-45. PMID: 21382272.
49. Aispuru-Lanche R, Jayo-Montoya JA, Maldonado-Martín S. Vascular-endothelial adaptations following low and high volumes of high-intensity interval training in patients after myocardial infarction. Ther Adv Cardiovasc Dis. 2024 Jan-Dec; 18:17539447241286036. DOI: https://doi.org/10.1177/17539447241286036.
50. Rusu ME, Bigman G, Ryan AS, Popa DS. Investigating the Effects and Mechanisms of Combined Vitamin D and K Supplementation in Postmenopausal Women: An Up-to-Date Comprehensive Review of Clinical Studies. Nutrients. 2024 Jul 20;16(14):2356. DOI: https://doi.org/10.3390/nu16142356.
51. Sugita M, Kapoor MP, Nishimura A, Okubo T. Influence of green tea catechins on oxidative stress metabolites at rest and during exercise in healthy humans. Nutrition. 2016 Mar;32(3):321-31. DOI: https://doi.org/10.1016/j.nut.2015.09.005.
52. Kim JH, Lee ME, Hwang SM, Lee JJ, Kwon YS. Association between Dietary Antioxidants and Atherosclerotic Cardiovascular Disease in South Korea: Insights from a Comprehensive Cross-Sectional Analysis. J Clin Med. 2024 Oct 11;13(20):6068. DOI: https://doi.org/10.3390/jcm13206068
53. Marotta F, Hayakawa K, Mikami Y, Morello P, Sugai M, et al. Relationship between gastrin cell number, serum, antral mucosa and luminal gastrin concentration and gastric acidity in antral atrophic gastritis. Gut. 1990 Mar;31(3):279-81. DOI: https://doi.org/10.1136/gut.31.3.279.
54. Wang PP, Luo ZG, Peng XC. Encapsulation of Vitamin E and Soy Isoflavone Using Spiral Dextrin: Comparative Structural Characterization, Release Kinetics, and Antioxidant Capacity during Simulated Gastrointestinal Tract. J Agric Food Chem. 2018 Oct 10;66(40):10598-10607. DOI: https://doi.org/10.1021/acs.jafc.8b00644.
55. Marotta F, O'Keefe SJ, Marks IN, Girdwood A, Young G. Pancreatic enzyme replacement therapy. Importance of gastric acid secretion,

H2-antagonists, and enteric coating. Dig Dis Sci. 1989 Mar;34(3):456-61.
DOI: https://doi.org/10.1007/BF01536271

56. Northoff H, Biesalski HK, Dickhuth HH, Fehrenbach E. Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of RRR-alpha-tocopherol supplementation. Antioxid Redox Signal. 2000 Spring;2(1):113-26.
DOI: https://doi.org/10.1089/ars.2000.2.1-113.
57. Khalili F, Vaisi-Raygani A, Shakiba E, Kohsari M, Dehbani M, et al. Oxidative stress parameters and keap 1 variants in T2DM: Association with T2DM, diabetic neuropathy, diabetic retinopathy, and obesity. J Clin Lab Anal. 2022 Jan;36(1): e24163. DOI: https://doi.org/10.1002/jcla.24163.
58. Chen QM. Nrf2 for protection against oxidant generation and mitochondrial damage in cardiac injury. Free Radic Biol Med. 2022 Feb 1; 179:133-143.
DOI: https://doi.org/10.1016/j.freeradbiomed.2021.12.001.
59. Ward AO, Sala-Newby GB, Ladak S, Angelini GD, Caputo M, Suleiman MS, Evans PC, George SJ, Zakkar M. Nrf2-Keap-1 imbalance under acute shear stress induces inflammatory response in venous endothelial cells. Perfusion. 2022 Sep;37(6):582-589.
DOI: https://doi.org/10.1177/02676591211012571.
60. Chaudhary MR, Chaudhary S, Sharma Y, Singh TA, Mishra AK, et al. Aging, oxidative stress and degenerative diseases: mechanisms, complications and emerging therapeutic strategies. Biogerontology. 2023 Oct;24(5):609-662.
DOI: https://doi.org/10.1007/s10522-023-10050-1.
61. Yu C, Xiao JH. The Keap1-Nrf2 System: A Mediator between Oxidative Stress and Aging. Oxid Med Cell Longev. 2021 Apr 19; 2021:6635460.
DOI: https://doi.org/10.1155/2021/6635460
62. Belka M, Gostyńska-Stawna A, Stawny M, Krajka-Kuźniak V. Activation of Nrf2 and FXR via Natural Compounds in Liver Inflammatory Disease. Int J Mol Sci. 2024 Oct 18;25(20):11213.
DOI: https://doi.org/10.3390/ijms252011213
63. Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, et al. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proc Natl Acad Sci U S A. 2004;101(10):3381–6. Epub 2004/02/27. 10.1073/pnas.0400282101
64. Fejes R, Pilat N, Lutnik M, Weisshaar S, Weijler AM, Krüger K, Draxler A, Bragagna L, Peake JM, Woodman RJ, Croft KD, Bondonno CP, Hodgson JM, Wagner KH, Wolzt M, Neubauer O. Effects of increased nitrate intake from beetroot juice on blood markers of oxidative stress and inflammation in older adults with hypertension. Free Radic Biol Med. 2024 Sep; 222:519-530.
DOI: https://doi.org/10.1016/j.freeradbiomed.2024.07.004.
65. Hernández-Cruz EY, Aparicio-Trejo OE, Hammami FA, Bar-Shalom D, Tepel M, Pedraza-Chaverri J, Scholze A. N-acetylcysteine in kidney disease: Molecular Mechanisms, Pharmacokinetics, and Clinical Effectiveness. Kidney Int Rep. 2024 Jul 20;9(10):2883-2903.
DOI: https://doi.org/10.1016/j.ekir.2024.07.020.
66. Kibreab S, Wang Z, Zhu X, Ren Y, Jing Y, Li X, Li L, Zhang B. Reciprocal REGγ-Nrf2 Regulation Promotes Long Period ROS Scavenging in Oxidative Stress-Induced Cell Aging. Oxid Med Cell Longev. 2023 Jan 10; 2023:4743885.
DOI: https://doi.org/10.1155/2023/4743885.
67. Mohammad Rahimi F, Sohrabi M, Saberi Kakhki A, Mohammad Rahimi N. Six Weeks to Wellness: The Role of Breathing Exercises Based on Motor Development Pattern in Improving Fitness and Quality of Life in Overweight Elderly Women. Biol Res Nurs. 2024 Oct 29:10998004241297403.
DOI: https://doi.org/10.1177/10998004241297403.
68. Zhang Q, Gou F, Shi P, Xu Z, Yan Z, He M, Yin X, He Y, Zhang J. Angiotensin-converting enzyme inhibitors provide a protective effect on hypoxia-induced injury in human coronary artery endothelial cells via Nrf2 signaling and PLVAP. Clin Hemorheol Microcirc. 2024;87(2):141-170.
DOI: https://doi.org/10.3233/CH-232007.
69. Ostrom EL, Traustadóttir T. Aerobic exercise training partially reverses the impairment of Nrf2 activation in older humans. Free Radic Biol Med. 2020 Nov 20; 160:418-432.
DOI: https://doi.org/10.1016/j.freeradbiomed.2020.08.016
70. Shimizu S, Mimura J, Hasegawa T, Shimizu E, Imoto S, et al. Association of single nucleotide polymorphisms in the NRF2 promoter with vascular stiffness with aging. PLoS One. 2020 Aug 11;15(8): e0236834.
DOI: https://doi.org/10.1371/journal.pone.0236834.
71. Shimoyama Y, Mitsuda Y, Hamajima N, Niwa T. Polymorphisms of Nrf2, an antioxidative gene, are associated with blood pressure in Japanese. Nagoya J Med Sci. 2014;76(1–2):113–20.
72. Sinha JK, Jorwal K, Singh KK, Han SS, Bhaskar R, Ghosh S. The Potential of Mitochondrial Therapeutics in the Treatment of Oxidative Stress and Inflammation in Aging. Mol Neurobiol. 2024 Sep 4. DOI: https://doi.org/10.1007/s12035-024-04474-0.
73. Sheng W, Yue Y, Qi T, Qin H, Liu P, Wang D, Zeng H, Yu F. The Multifaceted Protective Role of Nuclear Factor Erythroid 2-Related Factor 2 in Osteoarthritis: Regulation of Oxidative Stress and Inflammation. J Inflamm Res. 2024 Sep 21; 17:6619-6633. PMID: 39329083. DOI: https://doi.org/10.2147/JIR.S479186.
74. Ross D, Siegel D. The diverse functionality of NQO1 and its roles in redox control. Redox Biol. 2021 May; 41:101950.
DOI: https://doi.org/10.1016/j.redox.2021.101950.

75. Yuhan L, Khaleghi Ghadiri M, Gorji A. Impact of NQO1 dysregulation in CNS disorders. J Transl Med. 2024 Jan 2;22(1):4. DOI: https://doi.org/10.1186/s12967-023-04802-3.
76. Facchinetti MM. Heme-Oxygenase-1. Antioxid Redox Signal. 2020 Jun 10;32(17):1239-1242. DOI: https://doi.org/10.1089/ars.2020.8065.
77. Olivieri F, Biscetti L, Pimpini L, Pelliccioni G, Sabbatinelli J, Giunta S. Heart rate variability and autonomic nervous system imbalance: Potential biomarkers and detectable hallmarks of aging and inflammaging. Ageing Res Rev. 2024 Sep 27; 101:102521. DOI: https://doi.org/10.1016/j.arr.2024. 102521.
78. Agrawal N, Arya M, Kushwah P. Therapeutic voyage of synthetic and natural xanthine oxidase inhibitors. Chem Biol Drug Des. 2023 Nov;102(5):1293-1307. DOI: https://doi.org/10.1111/cbdd.14319.
79. Howden R, Gougian E, Lawrence M, Cividanes S, Gladwell W, et al. The influence of Nrf2 on cardiac responses to environmental stressors. Oxid Med Cell Longev. 2013; 2013:901239 Epub 2013 Apr 22. DOI: https://doi.org/10.1155/2013/901239.
80. Cavezzi A, Colucci R, d'Errico G. Mitoresilience: Hormesis, Psycho-physical Resilience, Mitochondria and Heart Rate Variability as Relevant Interplaying Elements in Longevity Medicine. Curr Aging Sci. 2023;16(1):25-32. DOI: https://doi.org/10.2174/1874609815666220606140754
81. Marotta F, Naito Y, Padrini F, Xuewei X, Jain S, et al.. Redox balance signalling in occupational stress: modification by nutraceutical intervention. J Biol Regul Homeost Agents. 2011 Apr-Jun;25(2):221-9.CC
82. Gounder SS, Kannan S, Devadoss D, Miller CJ, Whitehead KJ, et al. Impaired transcriptional activity of Nrf2 in age-related myocardial oxidative stress is reversible by moderate exercise training. PLoS One. 2012;7(9):e45697 Epub 2012/10/03. 10.1371/journal.pone.0045697
83. Waldman H. Astaxanthin Supplementation as a Potential Strategy for Enhancing Mitochondrial Adaptations in the Endurance Athlete: An Invited Review. Nutrients. 2024 Jun 3;16(11):1750. DOI: https://doi.org/10.3390/nu16111750.
84. Rastmanesh, R., Hejazi, J., Marotta, F., Hara, N. Type 2 diabetes: a protective factor for prostate cancer? An overview of proposed mechanisms. Clin Genitourin Cancer. 2014 Jun;12(3):143-8. DOI: https://doi.org/10.1016/j.clgc.2014.01.001
85. Nicholas-Okpara Viola A.N., Maryam Adegboyega, Julius Oben, Leonard Williams, Utazi Anastasia, et al. Exploring the Potential of Bioactive Compounds as Interventions for Dementia: Current Insights and Future Directions. Functional Foods Science, Vol. 4 No. 5 (2024): May 2024 DOI: https://doi.org/10.31989/ffs.v4i5.134
86. Jiawei (Vivian) Zhou, Danik Martirosyan. Functional foods for cholesterol management: A comparison between the United States and Japan. Functional Food Science, (4) 6 (2024): June DOI: https://doi.org/10.31989/ffs.v4i6.1372 2024
87. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. J Adv Res. 2021 Jul 6; 34:43-63. DOI: https://doi.org/10.1016/j.jare.2021.06.023.
88. Barreto R, Kawakita S, Tsuchiya J, Minelli E, Pavasuthipaisit K, et al. Metal-induced oxidative damage in cultured hepatocytes and hepatic lysosomal fraction: beneficial effect of a curcumin/absinthium compound. Chin J Dig Dis. 2005;6(1):31-6. DOI: https://doi.org/10.1111/j.1443-9573.2005. 00184.x
89. Martirosyan D, Lampert T, Lee M. A comprehensive review on the role of food bioactive compounds in functional food science. Functional Food Science Vol. 2 No. 3 (2022): March 2022 DOI: https://www.doi.org/10.31989/ffs.v2i3.906
90. Martirosyan D, Stratton S. Quantum and tempus theories of function food science in practice. Functional Food Science, Vol. 3 No. 5 (2023): May 2023. DOI: https://www.doi.org/10.31989/ffs.v3i5.1122