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MITOCHONDRIAL AND REDOX DYSFUNCTION IN POST-MENOPAUSE AS RISK FACTOR OF NEURODEGENERATIVE DISEASE: A PILOT STUDY TESTING THE ROLE OF A VALIDATED JAPANESE FUNCTIONAL FOOD

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During the menopause women may experience increased oxidative stress and decreased antioxidant capacity and, together with the decline of neurosteroids, this represents a risk factor for Alzheimer's disease. The aim of the present study was to test a functional food (FPP-ORI, Osato Research Institute, Gifu, Japan) on redox and mitochondrial efficiency in post-menopausal women. The study population consisting of 69 untreated post-menopausal women were given supplements as follows: Group A was given a multivitamin (MV) 1c 2 times a day, and group B was given FPP 4.5 g 2 times a day. Group C consisted of 23 fertile premenopausal women as the control group. The tests carried out on entry, and at 3 and 6 months were erythrocyte redox parameters, plasma oxidated proteins, brain-derived neurotrophic factor (BDNF) and peripheral blood mononuclear cell (PBMC) mitochondria cytochrome c oxidase Vmax activity. Menopausal women showed an increased malondialdehyde (MDA) ($p<0.05$ *vs* control) which was normalized by both treatments ($p<0.05$), but MV failed to do so in the BMI ≥26 subgroup ($p<0.05$). All other redox enzymes and BDNF were significantly lower in menopausal women and they responded only to FPP ($p<0.05$). Carbonyl protein level was higher in "BMI ≥26" subgroup ($p<0.05$) and reduced only by FPP ($p<0.05$). The PBMC cyclooxygenase to citrate synthase activity was reduced (< 40%) in the menopausal group ($p<0.01$) and only FPP caused a significant restoration ($p<0.05$). Although preliminary, these data confirm the redox and mitochondrial dysfunction occurring in post-menopause and responsive to FPP but very poorly to high dosage antioxidants. This may lead to potential preventive opportunities in menopause-associated neurodegenerative disease.

A growing body of experimental and clinical data supports the hypothesis that estrogens exert an essential role in regulating both the female brain and body metabolome. This is particularly relevant at a mitochondrial level in high energy-demanding organs such as brain and heart tissues (1-3). In this subcellular setting, estrogens enhance mitochondrial bioenergetic efficiency and protect from its dysfunction e.g., adenosine triphosphate (ATP) depletion and reduced membrane potential (4). Indeed,

Key words: menopause; redox dysfunction; mitochondria; FPP-ORI; MDA; GPx; SOD1; BDNF; COX activity

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within the brain, estrogen regulates glucose transport, aerobic glycolysis, and the bioenergetic system. Estrogen also modulates several enzymes of relevant importance in mitochondrial bioenergetics, such as glucose transporters, succinate dehydrogenase, pyruvate dehydrogenase, hexokinase, aconitase, alpha ketoglutarate dehydrogenase and Complexes I, III and IV involved in the electron transport chain (ETC) to produce ATP. This leads us to consider why the declining blood estrogen levels observed in menopause coincide with a similar event in brain bioenergetics eventually causing a metabolically impaired phenotype. It has to be recalled that under physiological cellular oxidative phosphorylation, mitochondria generate 1–4% reduced oxygen molecules which, by affecting ETC complexes I and III (5, 6), quickly react to form reactive oxygen species (ROS) (7). ROS are mostly produced in the mitochondria and these organelles are those most intensively damaged when an excess of oxidative stress is generated. Thus, an insufficient complex IV activity, unparalleled by a decline in activity of complexes I and III, as reported in Alzheimer disease (AD) patients, could lead to a relentless increased ROS spillover (8). These abnormalities are of interest considering that they have been clearly documented in AD while, with age progression, female gender represents the main risk factor for AD. Moreover, post-menopausal women show higher oxidative stress and reduced antioxidant capacity (9). As a matter of fact, estrogen receptors (ER) β are located within mitochondria and stimulate mitochondrial function and biogenesis. It has been demonstrated that hormone replacement therapy (HRT) decreases to a certain extent oxidative damage to both DNA and lipids in post-menopausal women (10), and lipid oxidation status may be inversely related to estrogen levels in this cohort of women.

MATERIALS AND METHODS

The aim of the present study was to test a functional food, i.e. Fermented Papaya Preparation (FPP-ORI, Osato Research Institute, Gifu, Japan) endowed with an established basic science and clinical validation on redox and immune system modulatory effectiveness as well as mitochondrial efficiency (11-14) in post-menopausal women. The FPP used in the present study was obtained from *Carica papaya L.* cultivated in selected non-GMO and organically-treated Hawaiian crops. The properly cleaned fruit was then further subjected for 10 months to a patented yeast fermentation process with pharmaceutical-grade batch-to-batch end-product control also by Electron Spin Resonance at the Osato Research Institute (Gifu, Japan). The final composition of FPP per 100 g is as follows: 90.7 mg carbohydrates, 37 mg glutamic acid, 27 mg aspartic acid, 18 mg leucine, 16.9 mg potassium, 16 mg arginine, 13 mg valine, 11 mg serine, 11 mg glycine, 11 mg phenylalanine, 9 mg tyrosine, 9 mg isoleucine, 8 mg proline, 8 mg treonine, 6 mg lysine, 5 mg hystidine, 5 mg methionine, 4.6 mg magnesium, 2.5 mg calcium, 240 µg niacin, 75 µg zinc, 17 µg vitamin B6, 14 µg copper, 2 µg folic acid and 2 mg tryptophan. Other claimed "fermented papaya" extracts available on the market were excluded since prior in-house electron spin resonance testing had ascertained their poor-to-nil antioxidant effect in biological systems (Mantello P.ORI Oxidative Stress Research Lab, Gifu, Japan. In-house data).

Study design and population

Sixty-nine post-menopausal non-smoker women who were not on HRT, bioidentical hormone replacement therapy (BHRT) or phytoestrogens, steroids, or ongoing food supplements were enrolled in the study. Participants were recruited using a variety of community-based approaches. The exclusion criteria for the study were: a major depression/anxiety disorder; excessive drinker (>7 drinks/week); illicit drugs user; past or present history of cancer; papaya fruit-allergy heart disease; autoimmune disorders; poor sleeper or suffering from sleep-apnea; attending mind-body relaxation programs. A preliminary selection had been previously carried out to exclude those subjects with Val66Met functional polymorphism of brain-derived neurotrophic factor (BDNF) (Fig. 1) since this has been found to be associated with an impaired release of BDNF (15).

The subjects were divided into two groups, matched for age, duration of menopause diagnosis, BMI, educational level (high-school diploma and higher degree), dietary intake and physical activity (kcal/week), while being instructed to refrain from intense physical exercise. For dietary intake, the web-based version of the National

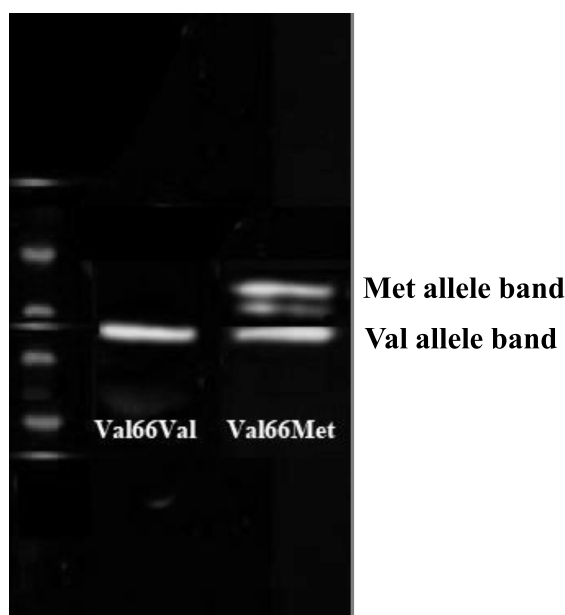

Fig. 1. *Western blotting characterization of BDNF genotype, Subjects showing Val66Met genotype were excluded (see text).*

Institute of Health Diet History Questionnaire (NIH DHQ) was adopted to assess diet history over the previous month and at each observation check point. The NIH DHQ is a food frequency questionnaire (FFQ) consisting of 124 food items and portion sizes.

A central allocation schedule for randomization was prepared through a computer-generated random sequence of the different treatment allocations. Treatment assignments were then made from separate randomization sequences created for each layer. Supplementation was carried out as follows: Group A was given a multivitamin (MV), (each capsule consisting of vitamin E 200IU, resveratrol 250 mg, coenzyme CoQ10 50 mg, pomegranate 100 mg, alpha-lipoic acid 100 mg,selenium 80 mcg) 1c 2 times a day, and group B was given FPP 4.5g 2 times a day, orally (sublingually) in the morning and approximately 2 h after lunch. Treatments were maintained for 6 months. Group C, consisting of 23 fertile premenopausal women (age 37-46), served as "young" controls. Essential clinical and anthropometric data are shown in Table I.

All procedures were approved by an independent Ethics Committee for non-pharmacological research (ReGenera Research Group for Aging Intervention, Milan, Italy). All subjects gave written informed consent.

Assays

The participants refrained from alcohol, vigorous exercise or NSAID use 72 h prior to fasting venous blood collection (50 mL).

The genomic DNA was extracted from 5 to 10 ml of peripheral whole blood from all participants, as well as purified from lymphocyte pellets according to GFX Genomic Blood DNA Purification kit (Bio-radLabolatories, Inc. Herules, CA, USA). The primer sequences used in the PCR analysis were as follows: APOE rs7412 F:ATGCCGATGACCTGCAGA R:ACTGGCGCTGCATGTCTT Product size (bp) 684; rs429358 F:TCGGAACTGGAGGAACAACTR: TACACTGCCAGGCGCTTCT Product size (bp) 260. Genomic DNA (50 ng) amplification comprised 30 cycles (94ºC for 5 min, 94ºC for 30 s, 65ºC for 30 s, and 72º for 30 s), followed by a cycle of 10 min at 72°C for the final chain extension. Moreover, for the analysis of restriction fragment length polymorphism, the amplification product was digested with HhaI, followed by 5% agarose gel electrophoresis at 110 V for 2 h and 30 min, staining with Gel Doc™ (Qiagen), and visualization of the DNA fragments under UV light,

Morning blood samples were taken at 7:00–8:30 a.m. to avoid potential confounding diurnal variation of oxidative stress biomarkers (16). On each examination day, at 1, 3 and 6 months, blood samples were taken for routine testing

Table I. *Baseline anthropometric, metabolic, family history and ApoE genotyping parameters*

Parameters/Groups at baseline	Premenopause	Menopause + MV	Menopause + FPP
Body weight (kg)	51.4±11.3	56.5±9.0	54.2±12.3
BMI (kg/m^2)	22±4.0	25±3.0	23±5.0
Glucose (mg/dl)	68.2±15.4	62.2±13.4	66.4±11.6
Family history of LOAD	3/23	6/35	7/34
ApoE4 carriers/non-carriers	4/23	9/35	8/39

LOAD: Late onset Alzheimer disease. There was no statistical difference among groups tested.

(glucose, transaminases, haemoglobin,urea, creatinine, LDL and HDL cholesterol, electrolytes) by automated standardized procedures (Hitachi 911 using commercial kits) and for further studies.

Erythrocyte parameters

Whole blood collected in lithium heparin was taken from each subject and kept in chilled vacutainers immediately after its withdrawal followed later by centrifugation at 1600 *g* at room temperature (500 g for 10 min) to only reticulocytes. These were treated with 10% acetic acid and then centrifuged to obtain erythrocyte pellets. The plasma and buffy layer were discarded, and the erythrocyte sediment was washed 3 times with isotonic saline (9g/L). A stock haemolysate (5 g Hb/dl) was then obtained by processing cells via three freeze-thaw cycles in dry ice and by adding five volumes of ice-cold distilled water. Cell membranes were retrieved by centrifugation, and the supernatant was immediately frozen until the measurement of glutathione peroxidase (GPx), Superoxide dismutase-1 (SOD1) and catalase (CAT) activities. The test cuvette contained 2 ml distilled haemolysate, while a further one contained 1 ml of buffer and 2 ml of haemolysate to serve as standard. The reaction was initiated by adding 8 ml of H_2O_2 (30 mM in the buffer) to the test tube with the extinction decay being calculated at 240 nm.

Lipid peroxidation was assessed as malonildialdehyde (MDA) by analyzing the levels of thiobarbituric acid reactive substances USING a colorimetric assay (λ=535 nm) (23). MDA was calculated according to the following formula: cell supernatant MDA (nmol/mg)=(Experiment group OD- blank control OD)/(Standard group OD- blank control OD)×Standard concentration/Sample concentration. Each group was repeated in triplicate. The results were expressed as nanomoles of MDA/mL (nmol/ mL).

Measurement of GPx activity was carried out as follows. Briefly, 0.02 ml of heparinised blood was treated with 0.1 ml of 5 mM GSH, 0.1 ml of 1.25 mM H_2O_2, 0.1 ml of 25 mM NaN3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37°C for 10 min. The reaction was halted by adding 2 ml of 1.65 % H_3PO_3 and the reaction mixture was centrifuged at 1500 rpm for 10 min; 2 ml of supernatant was used for the estimation according to the procedure given under tissue GPx determination. The result was expressed as U/g Hb.

In this method, GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized GSH is at once reduced via oxidation of nicotinammina adenina dinucleotid (NADH) to nicotinammide adenina dinucleotide fosfato (NADP). The decrease in absorbance at 340 nm was measured (Randox Kit) and the activity of GPx was calculated using the formula: Activity of GPx (IU/gHb) = GPx activity (IU)/ Hemoglobin (g/dl).

Superoxide dismutase (SOD) activity in red blood cell lysate was determined by employing xanthine and xanthine oxidase (Sigma; St. Louis, MO, USA) to generate superoxide radicals reacting with 2-(4-iodophenyl)-3-(4-nitrophenpl)-5phenyltetrazolium chloride to form a red formazan dye. The SOD-1 activity was then measured by the degree of inhibition of this reaction by adding xanthine oxidase at a concentration able to induce a 0.020% change in absorbance per minute at 550 nm (Randox). The activity of SOD was calculated by applying the following formula: Activity of SOD (IU/gHb) = SOD activity (IU)/ Hemoglobin (gr/dl) x 100.

Catalase activity in the erythrocytes lysate was measured by the kinetic method based on the disappearance rate of hydrogen peroxide (read at 240 nm) and expressed as k/g Hb, (or mU per g Hb). The inter- and intra-assay coefficients of variations (CV) were 2.9% and 6.6%, respectively.

Plasma/serum parameters

To assess the plasma concentration of advanced oxidation protein products (AOPPs) a commercial reagent kit (Thompson, Co., USA) was used following the manufacturer's instructions. The detection method makes 2-4-dinitrophenylhydrazine react with the protein carbonyl groups in an acidic milieu. Briefly, the oxidized protein standards were set by incubating bovine serum albumin (BSA) (50mg/ml) with 0.73 M H_2O_2 and 0.42 mM $Fe2^+$ at 37°C for 1 h. The reaction mixture was halted with 40 µM butylated hydroxytoluene and the carbonyl content of the oxidized BSA was analysed spectrophotometrically. Following a dilution with a solution of phosphate-buffered saline (PBS) and native BSA, a final carbonyl content was obtained of 2.0nmol/mg protein and protein concentration of 4mg/ml. A bicinchoninic acid kit (Sigma) enabled the assessment of total plasma protein levels. Following a derivatization step with DNPH, the plate was coated with 200 µl of sample and subjected to

overnight incubation at 4°C. The plate was washed with PBS/Tween (0.05% Tween 20 in PBS) and stopped with 0.1% BSA for 1.5 h. After a further washing passage, a dilution of 1:1500 with 0.1% BSA/0.1% Tween 20 in PBS of biotinylated primary anti-DNP antibody was prepared, incubated at 37°C for 1 h and subsequently added with streptavidin-biotinylated horseradish peroxidase conjugate. After incubation at room temperature for 1 h, the addition of tetramethyl benzidine (Sigma) enabled the color development and after 15–25 min incubation, the reaction was halted by H_2SO_4. The absorbance was read at 450 nm in a spectrophotometer (Genesis-20, Spectronic Inc. USA). Plasma AOPP concentration was calculated as µmolAOPPs/l plasma and each sample was assessed in triplicate and recorded an overall 11% of inter-assay coefficient of variation.

Serum levels of BDNF was quantitatively measured by a commercially available sandwich ELISA [BiosensisRapid™ Mature Brain-derived neurotrophic factor (BDNF)], according to the manufacturer's instructions, in 96-well plates by means of a specific human BDNF antibody. Briefly, the plates were coated with 100 µl of mouse anti-human BDNF antibody. Wells were washed three times and 100 µl of standards and samples were added and incubated for 2 h. Wells were then washed with buffer and 100 µl of biotinylated mouse anti-human BDNF antibody was added and incubated for 2 h. Afterwards, 100 µl of streptavidin-horseradish peroxidase was added for 20 min. Then, wells were washed, added with 100 µl of substrate and incubated for 20 min. The absorbance was read within 30 min in a microplate reader (SpectraMax Plus 384, Molecular Device), set at 450 nm, to determine BDNF concentrations in duplicate according to the standard curve, as previously designed by testing different concentrations. Samples were diluted between 1:100-1:150 and BDNF levels were then calculated by non-linear regression from the standard curves. Measurements were repeated in duplicate and value expressed in ng/ml after correcting for sample dilution. The sensitivity of the assay was 20pg/mL with intra-assay and inter-assay variations recorded at 6% and 10%, respectively.

Mitochondria cytochrome (PMC) c oxidase Vmax activity was measured in peripheral blood mononuclear cell (PBMC) with spectrophotometric and fluorometric assays. PBMC isolation was carried out soon after blood samples were diluted 1:1 using a balanced salt solution (anhydrous d-glucose 5.5 mM, KCl 5.4 mM, CaCl2 5 mM, MgCl2 0.98 mM, Tris 145 mM and NaCl 140 mM with pH adjusted to 7.6) and processed through density gradient centrifugation by a Histopaque® solution (density 1.077g/m, Sigma-Aldrich, USA). Each time of sampling, four 15 mL centrifuge cuvettes were employed to layer 16 mL of blood on 8 mL of Histopaque®. The suspension was centrifuged (30 min, 225 *g*) at room temperature and the layer containing the PBMCs at the interface was gently aspirated, washed in PBS (0.7 mM KH_2PO_4, 2.7mM KCl, 7.8 mM Na_2HPO_4, 136mM NaCl; pH7.4) and centrifuged again at 1,800 *g* for 5 min to remove any platelets. Afterwards, cell supernatants were discarded and the PBMC pellets were maintained at −80°C for further analysis. Enzymes were fully exposed to substrates by freezing and thawing the samples five times to yield optimal activities. Protein concentration was assessed using a DC protein assay kit (Bio Rad, Hercules, CA, USA). Cytochrome coxidase Vmax activity (COX, Complex IV, sec-1/mg) was calculated using standard spectrophotometric procedures in 0.5 ml reaction volume by measuring the oxidation of reduced cytochrome c at 550 nm. Cyclooxygenase (COX) activity was also referenced to its corresponding citrate synthase (CS) activity to correct for potential inter-sample differences in mitochondrial mass. CS Vmax activity (nmol/min/mg) was evaluated spectrophotometrically at 412 nm, monitoring the generation of 5-thio-2-nitrobenzoate after adding 100 µM oxaloacetate at 30°C.

RESULTS

Compared to "young" premenopausal controls, menopausal women, as a whole, showed significantly increased MDA concentration at the entry into the study ($p<0.05$, Table II). Starting from the first month of observation, the FPP group and both multivitamin (MV) and FPP treatment groups from the third month onward determined a statistically significant decrease of this parameter ($p<0.05$) of comparable extent. However, when looking at the subgroup of patients with BMI ≥26, it appeared that MV was ineffective in reducing MDA throughout the study period, whereas FPP yielded a statistically significant reversal to normal level from the third month onwards (Table III, $p<0.05$).

The levels of all other redox enzymes (SOD1, GPx and CAT) were significantly lower in menopausal women, irrespective of BMI, ApoE status and history of late-onset AD (LOAD) and they showed a statistically significant improvement only in FPP-supplemented subjects at the end of the observation period ($p<0.05$ *vs vs* MV-supplemented individuals, Table II).

Plasma levels of carbonyl protein in post-menopausal women showed a wide dispersion of data which did not bring to any statistically significant difference *versus* premenopausal women controls (Fig. 2). When the "BMI ≥26" subgroup was taken into account, it appeared that carbonyl protein levels in post-menopausal women were statistically higher than in fertile controls ($p<0.05$). Moreover, while MV intervention was ineffective, whatever observation period was tested, FPP brought about a significant decrement at 3- and 6-month testing ($p<0.05$, Table III).

Table II. *Time-course erythrocyte level of oxidative stress parameters in post-menopausal women: effect of MV and FPP*

Parameters (premenopause control value)	Group	Baseline	1 month	3 months	6 months
MDA nmol/ml (1.1± 0.2)	MV	2.6±0.2*	2.2±0.4	1.3±0.3**	1.3±0.4**
	FPP	2.4±1.3*	2.1±0.2§	1.1±0.2**	1.2±0.2**
SOD1 U/ mgHb (16.9± 1.2)	MV	9.5±2.2	9.7±3.2	10.2±2.5	10.6±1.9
	FPP	10.2±1.6	9.8±2.1	13.4±2.1§	16.3±1.5§
GPx U/ g Hb (57.8± 6.2)	MV	50.5±55.5	52.1±10.1	51.2±8.4	50.2±9.2
	FPP	49.5±9.5	50.4±11.5§	50.5±9.2§	57.4±11.4§
CAT U/mgHb (106.8± 9.2)	MV	78.8±9.5*	82.4±13.2	85.6±11.5	88.4±18.2
	FPP	81.2±10.5*	83.3±11.3§	92.8±15.2	108.2±13.6§

*MDA: Malonildialdehyde; SOD1: Superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; FPP: fermented papaya preparation. *Baseline values* vs *fertile premenopause control, $p<0.05$; § vs related multivitamin (MV) value, $p<0.05$; *** vs *baseline values, $p<0.05$.*

Table III. *Time-course plasma level of oxidative stress parameters in post-menopausal women: effect of MV and FPP*

(premenopause control value)	Group BMI ≥26	Baseline	1 month	3 months	6 months
MDA nmol/ml (1.1±0.2)	MV	2.1±2.3	2.2±0.8	2.2±0.6	1.9±0.4
	FPP	1.9±2.5	1.3±0.2	1.2±0.4*	1.2±0.5*
AOPPs µmol/l (30.1±8.2)	MV	36.1±12.3	35.2±8.8	35.6±9.6	36.9±9.4
	FPP	35.9±7.5	34.3±8.2	35.2±5.4	32.2±9.5*

*FPP: Fermented papaya preparation; MV: multivitamin; MDA: malonildialdehyde; AOPPs: oxidation protein products. ** vs *related MV and baseline values, $p<0.05$.*

Compared to the premenopausal women group, subjects in menopause showed a statistically lower plasma concentration of BDNF ($p<0.01$, Fig. 3). MV treatment did not affect this parameter when observed throughout the study period, whereas FPP yielded a trend improvement which reached a statistical significance at 3- and 6-months ($p<0.05$ *vs* baseline).

Overall, there was a great dispersion of data, not differentiating between hormonal status nor treatment employed (Table IV). However, by reading the values for COX/CS ratio, it appeared that, compared to the premenopausal control group,

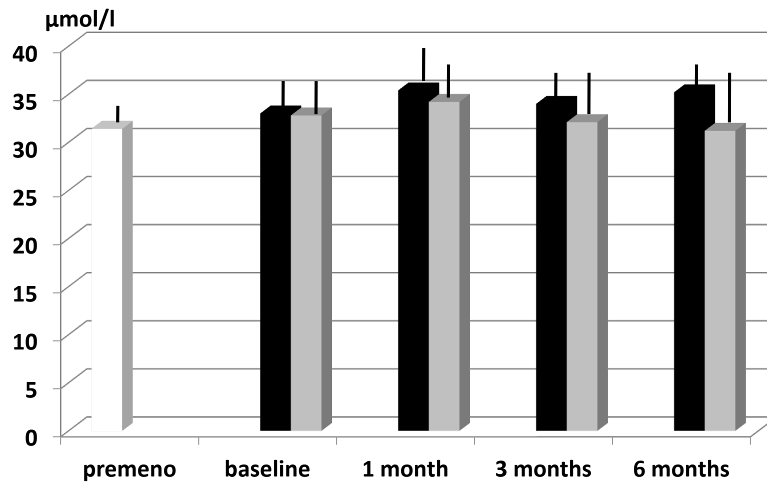

Fig. 2. *Plasma oxidation protein products (AOPPs) in premenopause women (premeno, white bar) and women under multivitamin (MV) (black bars) or fermented papaya preparation (FPP) (grey bars) supplementation. No significant difference among groups.*

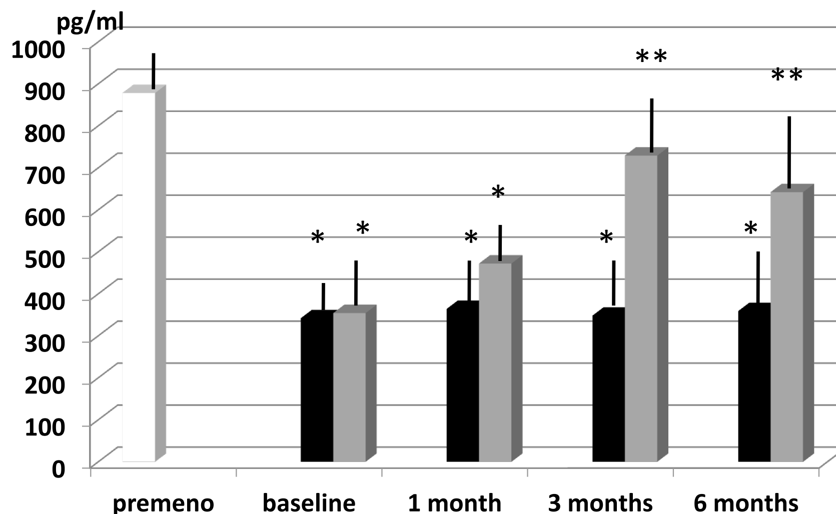

Fig. 3. *Plasma plasma brain-derived neurotrophic factor (BDNF) in premenopause women (premeno, white bar) and women under multivitamin (MV) (black bars) or fermented papaya preparation (FPP) (grey bars) supplementation. * $p<0.05$* vs *premenopausal women; *** vs *baseline and vs multivitamin (MV)-supplemented group.*

the total protein-referenced COX Vmax activity was reduced by more than 40% as an average in the menopausal group at baseline ($p < 0.01$). In particular, when we reanalyzed the time-course of COX/CS ratio after correction for age, such significant effect of FPP intervention was further confirmed (Fig. 4). On the other hand, BMI, ApoE status and history of LOAD did not affect further the significance (data not shown). MV treatment did not prove to be of any effect, unlike the FPP dietary intervention which yielded a significant restoration at 3- and 6-month observation (Table IV, $p<0.05$).

DISCUSSION

Although preliminary and ongoing extension

Table IV. *Time-course PBMC level of COX, CS and COX/CS ratio in post-menopausal women: effect of MV and FPP*

	Group	Baseline	1 month	3 months	6 months
COX sec^{-1}/mg	MV	34.8±22.1	35.5±21.8	36.6±20.4	35.9±21.4
(39.1±19.4)	FPP	36.9± 21.5	34.3± 17.2	37.8± 24.1	36.2± 19.8
CS nmol/min/mg	MV	139.7±24.2	138.7±29.5	140.5±21.3	141.2±31.3
(140.7± 26.7)	FPP	136.2± 22.6	134± 27.2	133.8± 31.2	135.7± 29.7
COX/CS ratio	MV	0.21±0.13*	0.23±0.11*	0.22±0.11*	0.22±0.14*
(0.31±0.11)	FPP	0.22±0.12*	0.26±0.15	0.30±0.16**	0.29±0.11**

FPP: Fermented papaya preparation; MV: multivitamin; COX: cycloxigenase; CS: citrate synthase.
* vs *premenopausal group value,* ** vs *baseline and vs MV-treated menopausal value.*

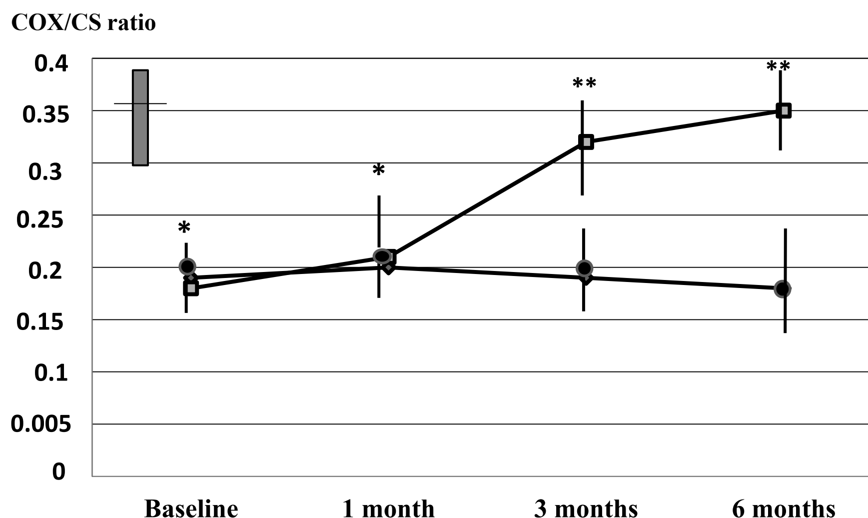

Fig. 4. *Time-course measurement of COX/CS ratio in PBMC adjusted by age. Squared dots: fermented papaya preparation (FPP) group, Round dots: multivitamin (MV) group. Upper left grey rectangle: control premenopausal mean value.* * vs *premenopausal group value,* ** vs *baseline and* vs *multivitamin (MV)-treated menopausal value.*

of the study is still in progress, these data confirm the reported redox imbalance taking place in post-menopausal women. This occurred whatever their BMI, ApoE status and possible family history of LOAD, although with some degree of association to metabolic and psychometric variables (17, 18). As a redox balance regulator, FPP showed to be significantly more effective than a multicomponent antioxidant formula as well as that reported by using red ginseng (19). These data are not new when considering prior double-blind or vitamin E-controlled clinical studies showing similar results (20, 21) through a well-documented nutrigenomic effect either in healthy young or elderly subjects (22). Overweight itself has been reported to be associated to oxidative stress whatever the age of women (23) and this may explain why only those women with BMI<26 were the ones with significantly higher AOPPs, given the wide scattering of values of this parameter in the pre- and post-menopausal groups. Interestingly, only FPP enabled a normalization of this parameter as well as of MDA which, in this subgroup, did not respond to multicomponent antioxidant supplementation. Indeed, besides a large body of experimental evidence, as pointed out by Taleb-Belkadi et al. (24), overweight has to be framed within a wider inflammatory/dys-metabolic disorder.

Although facing such phenomena requires a multimodal approach, a phytochemical intervention also endowed by brain protective properties has been envisaged (25). In this regard, the antioxidant/anti-inflammatory modulatory effect at gene expression level shown by FPP in the above-mentioned studies is likely to offer a more fundamental action. Of great relevance is also the finding that menopausal women, when analysed after age factor correction against the pre-menopausal group, showed a significant reduction of COX Vmax activity. These data seem to be in agreement with the recent findings reported by Mosconi et al. (26) in platelets of young subjects with family history of LOAD. Although our data as such cannot be held at the moment as endowed with a solid prognostic importance, it is of concern that a decrease in bioenergetic fluxes and mitochondrial enzyme activities together with mitochondrial number has been observed in Alzheimer's disease (42) and is envisaged to be a candidate for future interventions (27). Our population was not on hormone replacement therapy (HRT), and the intervention with either estrogen or selective β estrogen receptor agonists has been shown to offer some benefit on redox balance, although the data on protection against protein and lipid oxidation are conflicting (28-30). HRT is also known to improve the reported lower BDNF level, as also observed in the present study, and normalized by FPP supplementation. One can hypothesize that FPP-induced activation of Nrf2-mediated redox balance as well as of neurotrophic signaling pathway may prove to yield a potential preventive option in menopause at risk of developing neurodegenerative disease. Also, physical activity is known to bring about an overall protective effect in menopausal women. However, although an active lifestyle is known to induce positive antioxidant enzyme modulation, Farinha JB, et al. (31) have shown that physical activity may be unable to cause significant mitochondrial adaptations when measured by ETC enzymes. FPP has been shown to increase ATP and NADPH concentrations (18) as well as Rac2 gene transcription through increased Sp1 DNA binding activity in PBMC (32). However, molecular studies are still in progress. Given the above data and the very recently confirmed high safety profile of FPP (32), this nutraceutical supplementation offers an interesting opportunity within a wider health-promoting and disease-preventing strategy in menopausal women. A new study using this nutraceutical in post-menopausal women already on HRT is ongoing with the view of revealing any potential synergy.

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