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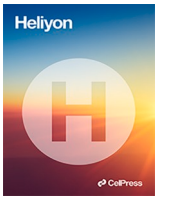

Research article

Beneficial effects of a combined lifestyle intervention for older people in a long-term-care facility on redox balance and endothelial function

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ABSTRACT

Objective: It has recently been highlighted how a short healthy life-style program (LSP) can improve the functional outcomes of older people admitted to a Long-Term Care (LTC) facility. Although it is known that life-style medicine-based interventions can exert anti-aging effects through the modulation of oxidative stress and mitochondrial function, the mechanisms underlying the aforementioned effects have not been clarified, yet. For this reason, in this study, the outcomes were focused on the investigation of the possible mechanisms underlying the benefits of a short LSP in older people. This was achieved by examining circulating markers of oxidative stress and immunosenescence, such as Tymosin β (Tβ4), before and after LSP and the effects of plasma of older people undergone or not LSP on endothelial cells.

Methods: Fifty-four older people were divided into two groups (n = 27 each): subjects undergoing LSP and subjects not undergoing LSP (control). The LSP consisted of a combination of caloric restriction, physical activity, and psychological intervention and lasted 3 months. Plasma samples were taken before (T0) and after LSP (T1) and were used to measure thiobarbituric acid reactive substances (TBARS), 8-hydroxy-2-deoxyguanosine (8OHdG), 8-Isoprostanes (IsoP), glutathione (GSH), superoxide dismutase (SOD) activity and Tβ4. In addition, plasma was used to stimulate human vascular endothelial cells (HUVEC), which were examined for cell viability, mitochondrial membrane potential, reactive oxygen species (ROS) and mitochondrial ROS (MitoROS) release.

Results: At T1, in LSP group we did not detect the increase of plasma TBARS and IsoP, which was observed in control. Also, plasma levels of 8OHdG were lower in LSP group vs control. In addition, LSP group only showed an increase of plasma GSH and SOD activity. Moreover, plasma levels of Tβ4 were more preserved in LSP group. Finally, at T1, in HUVEC treated with plasma

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from LSP group only we found an increase of the mitochondrial membrane potential and a reduction of ROS and MitoROS release in comparison with T0.
Conclusions: The results of this study showed that a short LSP in older persons exerts antiaging effects by modulating oxidative stress also at cellular levels. Implications of those findings could be related to both prognostic and therapeutic strategies, which could be pursued as antiaging methods.

1. Introduction

Aging arises from the oxidative damage to cells and tissues and presents significant increased risks of various aging-related pathological conditions, like neurodegenerative, cardiovascular, metabolic, skeletal muscle and immune system diseases. Many factors are involved in the onset and regulation of aging: genomic instability, epigenetic alterations, loss of proteostasis, altered autophagy, mitochondrial dysfunction, cellular senescence, etc [1].

Most of the above drivers have been associated with chronic oxidative stress caused by elevated levels of reactive oxygen species (ROS) and other oxidants release not balanced by antioxidants. It is to note that the resulting altered redox state is associated with aging and the progressive increase in the chance of morbidity and mortality, as well [2–5]. Among various markers of oxidative stress, the most extensively quantified in aging studies, as being associated to the acceleration of aging process, are thiobarbituric acid reactive substances (TBARS), 8-hydroxy-2-deoxyguanosine (8OHdG) and 8-Isoprostanes (IsoP). Instead, the most common evaluated antioxidants are glutathione (GSH) and superoxide dismutase (SOD) activity [6–10].

Considering the demographic shift, the identification of interventions aimed at promoting health and longevity, is mandatory in order to decrease the incidence and development of aging and aging-related diseases and, in turn, promote healthy aging and longevity [11–21]. In older people, several studies showed how personal lifestyle, including adequate physical activity, mental wellbeing, caloric restriction and nutritional intervention, could significantly increase longevity and mitigate the negative outcomes of chronic conditions [22,23]. About this issue, many proteomic analyzes and quantifications of oxidant and antioxidant markers have been conducted in elderly subjects [23], undergone or not undergone life-style interventions [6,24–26]. However, the available evidence is focused on community-dwelling older people [27], while it has been suggested that older subject living in long-term care (LTC) might present higher oxidative stress levels, with a lower antioxidant status, than general older population [28].

In our recent study, we have shown that in a population of older people admitted to a LTC facility, a relatively short period of healthy life-style program (LSP) obtained through combining moderate to high physical exercise, a low caloric diet and psychological wellbeing, was effective in terms of improvement of functional scores related to walk, balance, and disability [29].

As regarding the possible protective mechanisms, all the above interventions and in particular LSP, could modulate oxidative stress/redox state and inflammation and keep mitochondria function [30–35]. In particular, both caloric restriction and physical activity are reliable means of increasing lifespan, decreasing inflammation and ROS levels [26] and stimulating hormesis, which is a biological phenomenon where exposure to low doses of a stressor can induce adaptive responses resulting in improved health and longevity through increased production of the antioxidants and of the DNA repairing enzymes [36,37].

About mitochondria, it is well known that their dysfunction could be intimately linked to a wide range of processes associated with aging including senescence, inflammation, as well as, to the more generalized age-dependent decline in tissue and organ function [38–42]. For these reasons, the maintenance of an adequate mitochondrial function could play a pivotal role as an anti-aging tool. At this regard, it is to note that in a previous paper performed by using plasma of older people, we showed that changes of mitochondrial membrane potential of human vascular endothelial cells (HUVEC) were correlated with the patients' outcomes [43]. Those findings highlighted the existence of unknown circulating factors, which were able to mediate the damage to the endothelium and the crucial role played by endothelial cells in aging, as also suggested by others [44,45]. By this way, it could be hypothesized that the improvement of mitochondrial function in endothelial cells could be an additional mechanism involved in the beneficial effects we found in the subjects undergone the LSP.

Anyway, in spite of existing information regarding the role of life-style medicine-based programs on aging, data on the effect of a short period of LSP on oxidative stress and endothelial function are rather scarce, especially from the long-term care setting [27]. An interesting aspect could be also the one to verify whether LSP can exert anti-aging effects through the modulation of those unknown circulating factors, we previously found to be able to cause damages to endothelial cells. The results obtained could have interesting repercussions not only for the implementation of knowledge about the pathophysiology of aging and the protective role exerted by LSP, but also for diagnostic/prognostic purposes related to the evaluation of subjects' response to LSP and its maintenance over time. In addition, the in-depth study of the aforementioned aspects could lead to identification of mechanisms able to modulate the endothelial senescence and dysfunction, which could represent a promising therapeutic opportunity for the prevention of aging [44].

For this reason, in this study we aimed to evaluate plasma markers of redox state in older people living in a LTC facility, before (T0) and after a three-months-period of LSP (T1), which included physical exercise, diet and psychological wellbeing. In addition, we evaluated the effects of plasma of the subjects on HUVEC, in terms of cell viability, oxidants release and mitochondrial function.

2. Materials and methods

2.1. Participants selection

This study is a part of a large experimental study, which was performed according to the CONSORT Statement [28] and was approved on September 30th, 2020 by the Ethical Committee of the "Azienda Ospedaliera Maggiore della Carità" University Hospital in Novara (registration number: CE 232/20).

Eligible participants have been living in the "Belletti Bona" nursing home, which is a 144-beds LTC facility located in Biella (Piedmont, Italy; facility managed by the not-for-profit private company Anteo Impresa Sociale), for one or more years and requiring moderate or high nursing care [29]. We included in the study only subjects who were able to express their signed informed consent, according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). To keep the anonymity of sample data collection and analysis, an alphanumeric code was randomly assigned to each participant [29]. Before the intervention, baseline data was collected through clinical record screening. This included: demographic information, comorbidities, current therapy, use of tobacco, restraint use, performance in activities of daily living (adopting the Barthel Index (BI) [46] and the Katz ADL scales [47], and risk of falls (using the Tinetti scale [48]). The collection of such information is a routine activity of LTC facilities.

Sample size was estimated considering an improvement in functionality after the LSP, specifically an increase in the BI. The larger experimental study, of which this study is a part [29], was conducted using a pragmatic randomized controlled trial design. Pragmatic studies aim to assess the effectiveness of interventions in real-world settings; thus, we used the BI, a tool routinely employed in LTC facilities worldwide to assess patients' functionality. Considering a baseline BI score of 25 and aiming to detect a 15 % improvement (one-sided, 5 % significance level, 80 % power), we calculated a sample of 27 subjects per group, given an anticipated dropout rate of 10 % [49,50].

As described in our previous paper, the participants were randomly allocated to the intervention group (LSP), or control group. In the intervention group, subjects underwent a three-month multicomponent program. The LSP combined three components (namely physical exercise, healthy diet, and psychological wellbeing) to obtain a synergistic beneficial effect on the patients' functionality [29,51] and to decrease risk of falls [52]**.** While it has been suggested that physical exercise and a balanced nutrition might support healthy aging and reduce oxidative stress [53], the psychological wellbeing can increase adherence, motivation and self-confidence of patients toward the intervention, ultimately supporting the overall effectiveness [54]. Detailed description of each component is available in the supplementary materials of the primary study [29].

The physical exercise was organized into 45-min sessions, twice a week, requiring moderate to high effort to subjects and was managed by physiotherapists. The diet offered four different meals and was organized by nutritionists to provide fruits and vegetables and at least 30 g of proteins [55]. In order to reduce the risk of falls and hip fractures [56], daily calcium and vitamin D intake were managed to reach the minimum quantity suggested by the recommended daily intake levels [57,58]. Finally, the psychological wellbeing was delivered in weekly group sessions by experienced psychologists [29,43].

In contrast, patients in the control group received usual care. Specifically, their physical activity included physiotherapist-assisted walks lasting 5–10 min, five times per week. Additionally, residents typically participated in a weekly, low-intensity, 1-h group physical exercise session. Their diet consisted of normocaloric, normoproteic meals in accordance with local health authority regulations. Psychological support was provided through bi-weekly monitoring by a psychologist, with targeted interventions offered as needed.

Information regarding physical exercise and psychological wellbeing components (e.g., attendance rate) was collected from patients' clinical records, while food intake was monitored by health assistants during each meal. To ensure a controlled food intake, both participants and their families were asked to avoid any food supplementation. In addition, as a routine procedure of the facility, relatives are asked to report healthcare workers any additional food brought from outside, and the workers must report such events in the individual patient report. Moreover, participants were instructed to avoid additional and uncontrolled food intake. In all participants, blood samples were taken before (baseline, T0) and after the intervention, or after three months from T0 (T1).

2.2. Collection of blood samples

Blood samples were taken from participants, in the morning in fasting conditions by using BD Vacutainer tubes (sodium heparin as anticoagulant) on January 15th, 20th, and 24th, 2020 (baseline, T0) and on April 15th, 20th and 24th (T1). Each sample was immediately centrifuged by a refrigerated centrifuge (Eppendorf, mod. 5702 with rotor A-4-38) for 10 min, at 3100 rpm at 4 °C. The plasma obtained was divided into five tubes, which were kept at −80 °C at the Physiology Laboratory of the Università del Piemonte Orientale and further processed for the quantification of markers of redox state, as specified below, and for executing the *in vitro* experiments on HUVEC.

2.3. Plasma markers of redox state evaluation

2.3.1. Thiobarbituric acid reactive substances (TBARS) quantification

The evaluation of plasma TBARS was performed on each plasma sample by using the TBARS assay Kit (Cayman Chemical, Ann Arbor, MI, USA), which evaluates the malonyldialdeide (MDA) release [35–37] To do this, 100 μL of each plasma sample was added to sodium dodecyl sulphate solution (100 μL) and Color Reagent (2 mL). Each sample was boiled for 1 h and then shifted on ice for 10 min to stop the reaction. Thereafter, each sample was subjected to centrifugation (10 min at 1600×*g*, 4 °C); after that, 150 μL was

transferred to 96-well plates for MDA detection through a spectrophotometer (VICTOR™ X Multilabel Plate Reader, PerkinElmer; Waltham, MA, USA) at excitation/emission wavelengths of 530–540 nm. In order to quantify the correct value of TBARS in each sample (expressed as MDA in μM), a reference standard curve with the TBARS Standard was prepared. Each measurement was performed in triplicate. Detection Range: 0.625–50 μM.

2.3.2. 8-Hydroxy-2-deoxyguanosine (8OHdG), 8-Isoprostanes (IsoP) and Thymosin beta (Tβ4) quantification

Plasma 8OHdG, IsoP and Tβ4 measurements were performed by using specific ELISA assays (8-hydroxy-2-deoxyguanosine ELISA Kit; Prodotti Gianni srl, Milan, Italy; 8-Isoprostanes ELISA Kit; Labospace Milan, Italy; Human TMSβ4 ELISA Kit; Wuhan Fine Biotech Co.; Wuhan, China) [4]. In order to evaluate 8OHdG, 50 μl of each plasma sample, the standard solution and 50 μl of 8-hydroxy-2--deoxyguanosine Antibody Preparation were added to each well and then the plate was incubated at room temperature (RT) for 1 h. After 1 h, each well was washed 4 times with 300 μl of 1X Wash Buffer and then, 100 μl of Substrate Solution (tetramethyl-benzidine) was added to each well and incubated for 30 min at RT in the dark. The enzyme reaction was stopped by adding 100 μl of Stop Solution (1 M phosphoric acid) into each well and the plate was read immediately. Assay Detection Range: 1.563–100 ng/mL. Sensitivity <0.94 ng/mL. Inter-assay and intra-assay <5.

As regarding IsoP measurement, 1 ml of plasma (adjusted with 12 μl acetic acid to pH 4) and 1 ml of ethyl acetate were combined and centrifuged at 2000 rpm for 10 min at RT. By this way three phases were formed: the upper organic phase (ethyl acetate phase containing lipoproteins), the interphase (containing proteins) and a lower phase (aqueous phase). The organic phase was collected and transferred in a new tube. The interphase was discarded, and the lower phase was transferred in a new tube and used for repeating the acetate extraction for three times. Subsequently, the organic phase was evaporated in a Speedvac. The dried residues were dissolved in 2 ml of 20 % KOH solution and incubated for 1 h, at 50 °C. The 2 ml of the aqueous solution was diluted with 3 ml of H2O and pH was adjusted using 20 % formic acid. Two ml of ethyl acetate were added and centrifuged at 2000 rpm for 10 min at RT. The procedure was repeated for three times. The ethyl acetate in the upper phase was dried in a Speedvac. For the ELISA assay, the dried sample-residue was dissolved in 20 μl ethanol and 130 μl of 1X Sample Dilution Buffer. For the competitive 8-Isoprostanes ELISA assay, 150 μl of sample was further diluted 1:16 with a final pH 7,4. At the end of samples preparation, 200 μl of 1X Sample Dilution Buffer was added into the blank wells, while 100 μl of 1X Sample Dilution Buffer was added into maximum binding control wells. One hundred μl of each sample and Standard were added into appropriate wells. Furthermore, 100 μl of the 1X-Horseradish Peroxidase (HRP) conjugate was added in each well, except in the blank control wells. The plate was incubated at RT for 2 h. After this time, the plate was washed 3 times with 400 μl of 1X Wash Buffer and then, 200 μl of tetramethyl-benzidine was added to all of the wells and incubated for 30 min at RT. The enzyme reaction was stopped by adding 50 μl of Stop Solution (2 N sulfuric acid) and plate was read immediately. Assay Detection Range: 15.625–1000 pg/ml. Sensitivity: 9.375 pg/ml. Intra-Assay: CV < 8. Inter-Assay: CV < 10.

For Tβ4 measurement, the samples were diluted accordingly with the provided sample diluent and plate was washed 5 times. For the experiments, 50 μl of the standard, blank or sample were added to appropriate wells. The blank was added with 50 μl sample/dilution buffer. Then, 50 μl of Biotion-labeled Antibody Working solution was added to each well and incubated for 45 min at 37 °C. Then, the plate was washed 3 times with washing buffer. 100 μl of HRP-Streptavidin Conjugate was added to each well and incubated for 30 min at 37 °C. Again, washing of plate was carried out for 5 times with washing buffer. We added 90 μl of Tetramethylbenzidine Substrate to each well and incubation was performed for 10–20 min in dark at 37 °C. Thereafter, 50 μl of stop solution was added to each well. Assay Detection Range: 31.25–2000 pg/ml. Sensitivity: 18.75 pg/ml. Intra-assay: CV < 6. Inter-assay: CV < 5.

The 8OHdG, IsoP and Tβ4 were detected following the manufacturer's instructions through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), using a wavelength of 450 nm. The value of each sample was quantified in respect to 8OHdG and IsoP standard curves. 8OHdG and Tβ4 were expressed as ng/ml, whereas IsoP was expressed as pg/ml. Each measurement was performed in triplicate.

2.3.3. Glutathione (GSH) quantification

GSH measurement was performed in plasma through the Glutathione Assay Kit (Cayman Chemical) [43,59] [43,59–61]. Briefly, each plasma sample was deproteinated by adding *meta*-phosphoric acid solution in an equal volume. After centrifugation at 2000*g* for 2 min, the supernatant of each sample was collected and 50 μL/mL of TEAM reagent was added to increase the pH. A total of 50 μL sample was moved to 96-well plates where the measurement of GSH was executed through a spectrophotometer (VICTOR™ X Multilabel Plate Reader) by using excitation/emission wavelengths of 405–414 nm. To perform an accurate GSH quantification (as μM), a reference curve was prepared by using the GSH Standard. Each measurement was performed in triplicate. Assay Detection Range: 0.5–16 μM.

2.3.4. Superoxide dismutase (SOD) activity quantification

The SOD activity was determined in plasma by using the Superoxide Dismutase Activity Assay Kit (Prodotti Gianni srl) [7]. For the experiments, 20 μl of each plasma sample (diluted 1:5) or standard were added in each well, together with 200 μl of WST working Solution and 20 μl of Enzyme Working Solution. In the blank 1 well, 10 μl of double-distilled water (ddH2O), 200 μl of WST working Solution and 20 μl of Enzyme Working Solution were added. In the blank 2 well, 20 μl of each plasma sample (diluted 1:5) or standard were added in each well, together with 200 μl of WST working Solution and 20 μl of Dilution Buffer. Finally, in the blank 3, 20 μl of ddH2O, 200 μl of WST working Solution and 20 μl of Dilution Buffer, were added. The plate was left on a shaker for 5 min and then, 20 μl Stop solution was added in each well. After incubation at 37 °C for 20 min, the fluorescence was read by using a spectrophotometer (VICTOR™ X Multilabel Plate Reader), with a wavelength of 450 nm. In order to quantify SOD activity (%), a reference standard value prepared by using xanthine oxidase was used. Each measurement was performed in triplicate. The assay measures SOD activity down

to 0.005 U/ml.

2.4. In vitro experiments

2.4.1. Culture of HUVEC

HUVEC were purchased from ATCC (catalog. no. CRL-1730TM) and were maintained in Kaighn's Modification of Ham's F-12 Medium (F–12K Medium; ATCC; catalog.no. 30-2004TM), containing 2 mM L-glutamine (Euroclone S.p.A.; Pero, Milan, Italy), 1500 mg/L sodium bicarbonate (Euroclone), and supplemented with 0.1 mg/ml heparin (Merck Life Science srl, Milan Italy), 100 μg/ml endothelial cell growth supplement (ECGS; Merck Life Science), 1 % penicillin and streptomycin and 10 % foetal bovine serum (FBS; Euroclone).

To evaluate the effects of plasma samples taken from the subjects on cell viability (MTT Assay), mitochondrial membrane potential (JC-1 Assay), mitochondrial reactive oxygen species (ROS) (MitoROS assay) and ROS (DCFDA-Cellular ROS Detection Assay kit) release on HUVEC, co-culture experiments were performed, by using specific Transwell inserts, as previously performed (Fig. 1; [43, 61]). Experiments were conducted in triplicate and repeated at least three times.

For the experiments, plasma samples were plated in the apical compartment of the insert and left to act for 3 h, while, HUVEC were plated in the basal compartment. Experiments were performed with 10 % plasma calculated in relation with total volume of each insert as previously performed [43,61]. Some cell samples were not treated with plasma and were used as control. After 3 h stimulation with plasma, the inserts were removed and various assays were performed in triplicate and analyzed by using a spectrophotometer.

2.4.2. Cell viability

Cell viability was examined in HUVEC by using the 1 % 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT; Life Technologies Italia, Monza, Italy) dye. For the experiments, 50000 HUVEC cells/well were plated in 24-Transwells plates in complete medium [35,38–40]. HUVEC were treated with 10 % plasma for 3 h. After each treatment, the medium was removed, and fresh culture medium without red phenol and FBS and with 0.5 mg/ml MTT dye was added to the 96-well plates containing the cells and incubated for 2 h at 37 °C in an incubator. Thereafter, the medium was removed, and a MTT solubilization solution (dimethyl sulfoxide; DMSO; Merck Life Science) was added and mixed in a gyratory shaker until the complete dissolution of formazan crystals. Cell viability was determined by measuring the absorbance through a spectrometer (VICTOR™ X Multilabel Plate Reader) with a wavelength of 570 nm and cell viability was calculated by setting control cells (non-treated cells), as 100 %.

2.4.3. Mitochondrial membrane potential measurement

Mitochondrial membrane potential was measured in HUVEC with JC-1 assay. For the experiments, 50000 HUVEC cells/well were plated in 24-Transwells plates in complete medium and were treated as described for cell viability. After stimulations, the medium of cells plated in starvation medium was removed and cells were incubated with 5,51,6,61-tetrachloro-1,11,3,31 tetraethylbenzimidazolyl carbocyanine iodide (JC-1) 1X diluted in Assay Buffer 1X for 15 min at 37 °C in an incubator, following the manufacturer's instruction (Cayman Chemical; [61–64]. After incubation with plasma as above described, the cells were washed twice with Assay Buffer 1X and then the mitochondrial membrane potential was determined by measuring the red (excitation 550 nm/emission 600 nm) and green (excitation 485 nm/emission 535 nm) fluorescence through a spectrometer (VICTOR™ X Multilabel Plate Reader). The data were normalized versus control cells (non-treated cells).

2.4.4. ROS release quantification

The oxidation of 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA) into 2,7-dichlorodihydrofluorescein (DCF) was used to assess ROS generation, following the manufacturer's instructions (Cayman Chemical; 38, 40). For the experiments, 50000 HUVEC cells/insert were plated in 24-Transwells plates in complete medium, following the same experimental protocol followed for MTT and

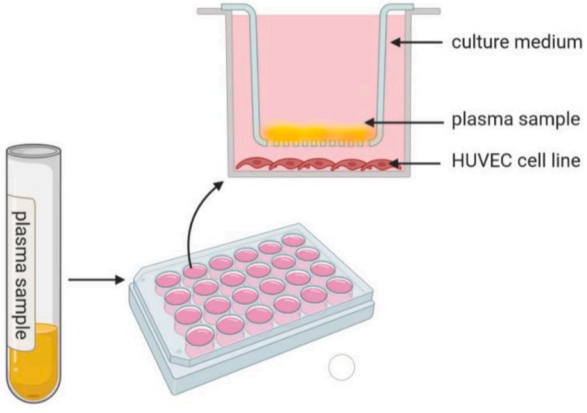

Fig. 1. Transwell inserts.

JC-1. Briefly, after treatments, the reactions were stopped by removing the medium and washing with phosphate buffer saline (PBS) followed by staining with 10 μM H2DCFDA for 20 min at 37 °C. The fluorescence intensity of DCF was measured at an excitation and emission wavelength of 485 nm and 530 nm, respectively, by using a spectrophotometer (VICTOR™ X Multilabel Plate Reader). Results were expressed as DCF fluorescence intensity, which was proportional to the amount of intracellular ROS. The data were normalized versus control cells (non-treated cells).

2.4.5. MitoROS release quantification

The mitoROS production was determined through the Mitochondrial ROS Detection Assay Kit (Cayman Chemical; [43,61,63,65]. Briefly, 50000 HUVEC cells/insert were plated in 24-Transwells plates in complete medium, and then the same experimental protocol followed for MTT, JC-1 and ROS assays was used. After the treatment, the reactions were stopped by removing the culture media and addition of 120 μl of Cell Based Assay Buffer. After that, the Buffer was aspirated and 100 μl of Mitochondrial ROS Detection Reagent Staining Solution, was added in each well and incubated at 37 °C, protected from light for 20 min. After the stimulation, the Staining Solution was removed and each well was washed with 120 μl of PBS for three times. The mitoROS production was measured with an excitation and emission wavelength of 480 nm and 560 nm, respectively, by using a spectrophotometer (VICTOR™ X Multilabel Plate Reader; PerkinElmer). The data were normalized versus control cells.

2.5. Statistical analysis

The normality of distribution of continuous variables was tested by Shapiro–Wilk test. The results obtained as concerning plasma oxidants/antioxidants and the *in vitro* experiments were expressed as median ± range and were examined through the Mann-Whitney T test, while Fisher's exact test was used for categorical variables. The statistical analysis was executed with GraphPad Prism (version 9.0; GraphPad Software; San Diego, CA, USA). A value of $p < 0.05$ was considered as statistically significant.

3. Results

Baseline characteristics of participants are shown in Table 1 (adapted from: Conti et al., 2024 [29]).

At T0, LSP group and control group did not differ in terms of age, gender, BMI, comorbidities, use of medications, care intensity level and psychophysical function scores. Moreover, the two groups were not significantly different in terms of performance in activities of daily living and risk of falls. Also, LSP and control groups had similar calcium intake and the vitamin D plasma levels did not differ at T0 or T1 (Table 2). Physical exercise was attended by all the subjects of the LSP group, with an average participation of 9 out of the 13 total sessions. The LSP group showed a lower energy intake than the control group. As regarding plasma oxidants/antioxidants measurements, the subjects of LSP and control group did not differ at T0, as reported in Table 2. Instead, it is to note that at T1 we observed statistically significant differences between LSP group and control group (Figs. 2 and 3; Table 2).

Hence, we found an increase of some markers of oxidation, like TBARS and IsoP at T1 in control group, only ($p < 0.05$; Fig. 2 A, B, C, D; Table 2. Instead, the levels of 8OHdG were reduced both in LSP group and control group, at T1, although at a lesser extent in control group (−20 % in control group: from 46 ng/ml of T0 to 36 ng/ml of T1;-62 % in LSP group: from 49 ng/ml of T0 to 18 ng/ml of T1; $p <$

Table 1
Baseline characteristics of participants allocated to LSP group vs control group.

	Control group (n = 27)	LSP group (n = 27)	Test	p-value
Demographic				
Age at enrollment (years, mean, SD)	83.26 (9.10)	84.85 (7.87)	MWU	0.78
Gender (female, n, %)	17 (62.96)	22 (81.48)	F	0.22
Weight (kg, SD)	59.68 (16.17)	63.36 (13.26)	MWU	0.26
Body mass index (kg/m^2, SD)	22.27 (4.83)	23.98 (5.01)	MWU	0.24
Tobacco use				
Never (n, %)	19 (70.4)	16 (59.3)	MWU	0.18
Ex-smoker (n, %)	4 (14.8)	2 (7.4)		
Current smoker (n, %)	4 (14.8)	3 (11.1)		
Unknown (n, %)	0 (0)	6 (22.2)		
Health				
Comorbidities (n, SD)	5.85 (2.68)	7.11 (3.27)	MWU	0.16
Number of drugs (n, SD)	7.48 (2.85)	8.30 (3.27)	MWU	0.45
Use of restraints (daily use, n, %)	12 (44.44)	10 (37.03)	F	0.78
Care Intensity Level (SD)	8.30 (2.03)	7.85 (2.16)	MWU	0.39
Scales				
Barthel index (SD)	30.85 (28.44)	36.89 (27.73)	MWU	0.25
Tinetti Score - walk (SD)	4.93 (4.39)	7.04 (3.63)	MWU	0.09
Tinetti score - balance (SD)	4.96 (4.78)	7.44 (4.23)	MWU	0.06
Tinetti score (SD)	9.89 (9.02)	14.48 (7.67)	MWU	0.08
Katz ADL (SD)	1.93 (2.20)	2.67 (2.35)	MWU	0.20
Energy intake (Kcal, SD)	1615 (152)	1486 (217)	MWU	0.51
Calcium intake (mg, SD)	828 (211)	707 (282)	MWU	0.10

MWU: Mann-Whitney-U test; F: Fisher's exact test. Adapted from: Conti et al., [29].

Table 2
Plasma levels of oxidants/antioxidants and vitamin D in LSP group and control group.

	LSP group T0	Control group T0	LSP group T1	Control group T1	p- value
TBARS (μM)	17 (10–32)	17 (8–33)	18 (8–30)	30 (13–48)*	<0.05 *T1 No LSP vs T1 LSP
IsoP (pg/ml)	534 (376–626)	523 (315–580)	565 (410–603)	670 (505–734)*	<0.05 *T1 No LSP vs T1 LSP
8OHdG (ng/ml)	49 (35–57)	46 (34–51)	18 (8–35)	36 (17–40)*	<0.05 *T1 No LSP vs T1 LSP
GSH (μM)	2.6 (1.4–3.6)	2.7 (2–3)	6.3 (4.5–6.8)	3 (2–3.1)*	<0.05 *T1 No LSP vs T1 LSP
SOD activity (%)	50 (38–59)	49 (37–62)	59 (50–65)	32 (19–45)*	<0.05 *T1 No LSP vs T1 LSP
Tymosin β (ng/ml)	1277 (950–1512)	1257 (832–1556)	770 (519–797)	480 (400–577)*	<0.05 *T1 No LSP vs T1 LSP
25OHD (ng/ml)	30.14 (28–32.25)	30.21 (27.76–32.66)	41.53 (41.31–41.75)	40.68 (40.34–41.01)*	<0.05 *T1 No LSP vs T1 LSP

TBARS = thiobarbituric acid reactive substances; IsoP = 8-Isoprostanes; 8OHdG = 8-hydroxy-2-deoxyguanosine; GSH = glutathione; SOD = superoxide dismutase; 25OHD: Vitamin D. LSP = older people subjected to LSP; Control = older people not subjected to LSP. LSP = healthy life-style program. The results are expressed as median and range of three different measurements.

0.05; Fig. 2 E, F; Table 2).

As far as plasma antioxidants are concerned, only in LSP group we showed an increase in GSH levels at T1 (Fig. 3. B; Table 2). Instead, it should be emphasized that the plasma SOD activity increased at T1 only in LSP group, while in control group it decreased compared to what was observed at T0 (Fig. 3 C, D; Table 2). It is also to note that we observed a smaller reduction of the plasma levels of Tβ4 in LSP group at T1 vs T0, in comparison with control group (−62 % in control group: from 1257 ng/ml of T0 to 480 ng/ml of T1 -37 % in LSP: from 1277 ng/ml of T0 to 770 ng/ml of T1; $p < 0.05$; Fig. 3 E, F; Table 2).

It can also be observed that at T1, the percentage changes in plasma values of markers of oxidation, such as TBARS, IsoP and 8OHdG compared to T0, were significantly greater in control group versus LSP group (Fig. 4 A-C). Instead, the percentage changes of plasma GSH levels, SOD activity and plasma Tymosin β levels were significantly lower in the control group vs LSP group, at T1 vs T0 (Fig. 4 D-F).

The best redox balance shown in the plasma of the intervention group participants was confirmed by *in vitro* experiments carried out by stimulating the HUVEC with plasma.

As observed for the redox state parameters, also as regarding cell viability, mitochondrial membrane potential, mitoROS and ROS release, no significant differences were observed in baseline values of HUVEC, which were, thereafter, stimulated with plasma of LSP group and control group at T0 (Table 3).

Instead, the mitochondrial membrane potential of plasma-treated HUVEC of the LSP group at T1 was increased in comparison with what was observed at T0. On the other hand, it was reduced in the plasma-treated HUVEC of the control group (Fig. 5; Table 3).

That the mitochondrial function was improved in HUVEC treated with plasma of the LSP group was confirmed by the analysis of ROS and mitoROS release, as well. Hence, both ROS and mitoROS release were reduced at T1 vs T0, in the LSP group only. Instead, an increase of mitoROS release was found at T1 in the control group (Fig. 6.; Table 3).

Finally, we observed that the treatment of HUVEC with plasma of the two groups had different effects on the viability of HUVEC. Hence, in HUVEC treated with plasma of the control group cell viability was reduced at T1 vs T0, whereas it was increased in HUVEC treated with plasma of the LSP group (Fig. 7; Table 3).

As shown in Fig. 8A and B, the percentage changes of cell viability and mitochondrial membrane potential of HUVEC treated with plasma of control group were lower at T1 vs T0 than those found in LSP group. Instead, the percentage changes of MitoROS and ROS release were significantly higher in HUVEC treated with plasma of control group vs LSP group (Fig. 8 C, D).

4. Discussion

The results of this study performed in a group of older people undergoing a multicomponent program, which combined physical exercise, healthy diet, and psychological wellbeing showed an improvement in plasma parameters of oxidative stress, with particular reference to the antioxidants. Also, maintenance of mitochondrial function, which was accompanied by a reduction of the oxidants release, was observed in HUVEC treated with plasma of LSP subjects.

Our findings could be of particular interest considering that aging is a relatively new problem worldwide and that the identification of strategies to maintain well-being in older age is crucial [22].

It is well known that environment and life-style, including factors like diet and physical activity, play a key role in healthy aging. Although issues should be addressed at this regard, both physical exercise and nutritional interventions could exert their beneficial effects by counteracting negative effects and toxicity of oxidative stress on health and improving antioxidant defenses [66].

Also, physical activity and caloric restriction have been considered as “hormetic” stressors and studied for their “hermetic” effects in aging [36]. By this way, those factors could activate cellular mechanisms that enhance resilience and resistance to more severe stressors, through multiple mechanisms, including improving mitochondrial function [67].

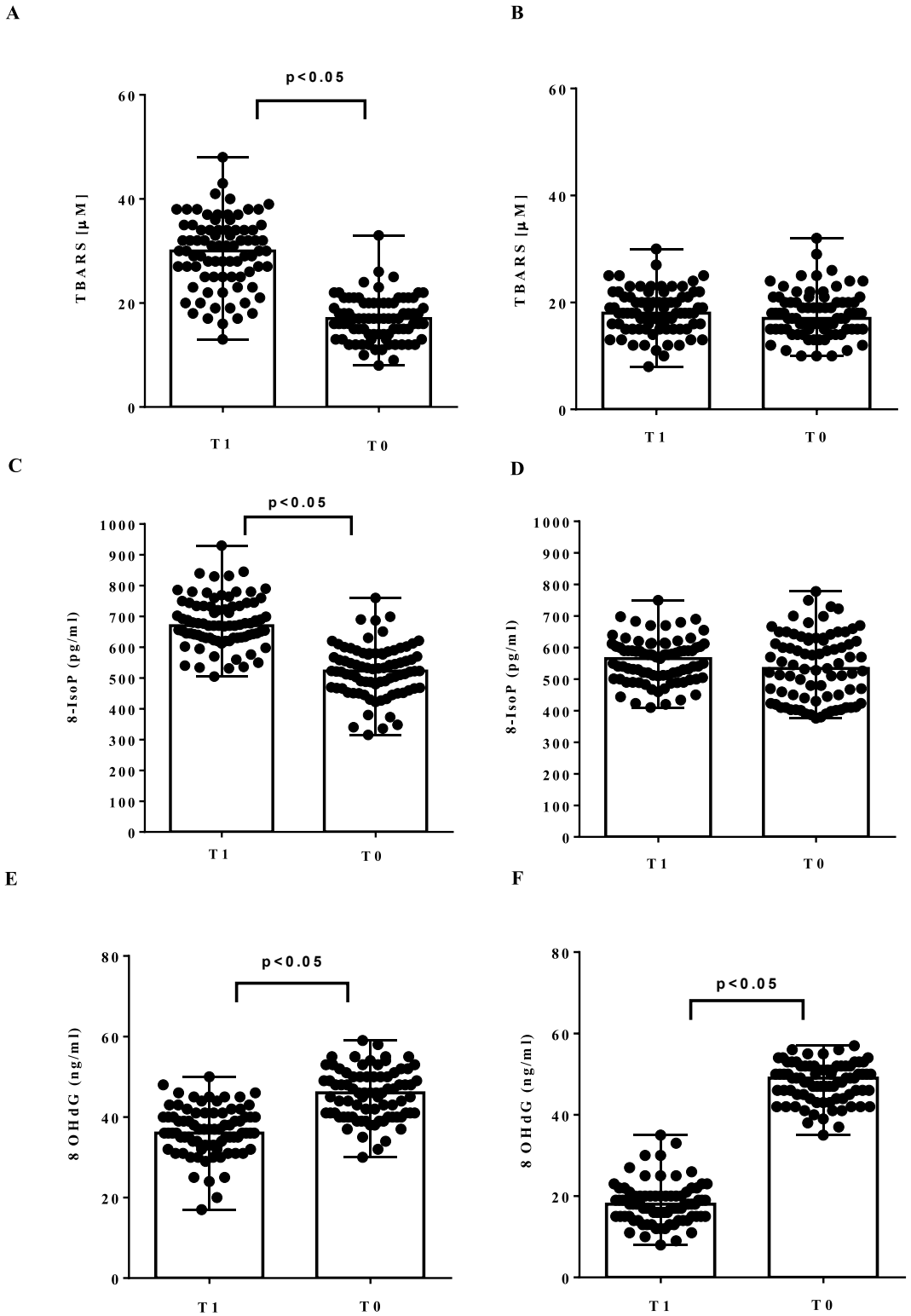

Fig. 2. Plasma levels of thiobarbituric acid reactive substances (TBARS; A, B), 8-Isoprostanes (IsoP, C, D), and 8-hydroxy-2-deoxyguanosine (8OHdG; E, F) in older people subjected to LSP (B, D, F) versus older people not subjected to LSP (A, C, E). The results are expressed as median and range of three different measurements. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

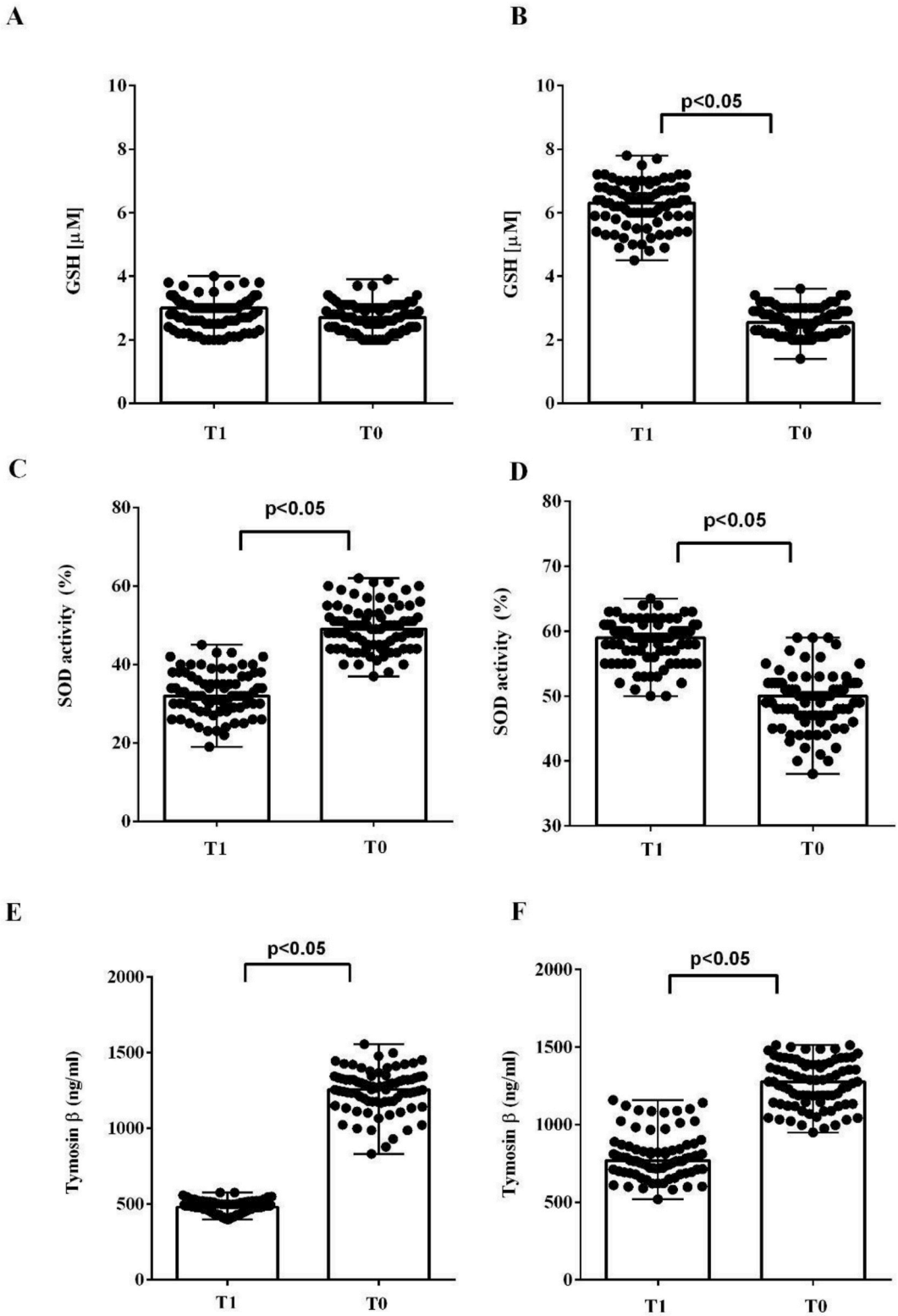

Fig. 3. Plasma levels of glutathione (GSH) (TBARS; A, B), superoxide dismutase activity (SOD; C, D), and Thymosin beta (E, F) in older people subjected to LSP (B, D, F) versus older people not subjected to LSP (A, C, E). Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

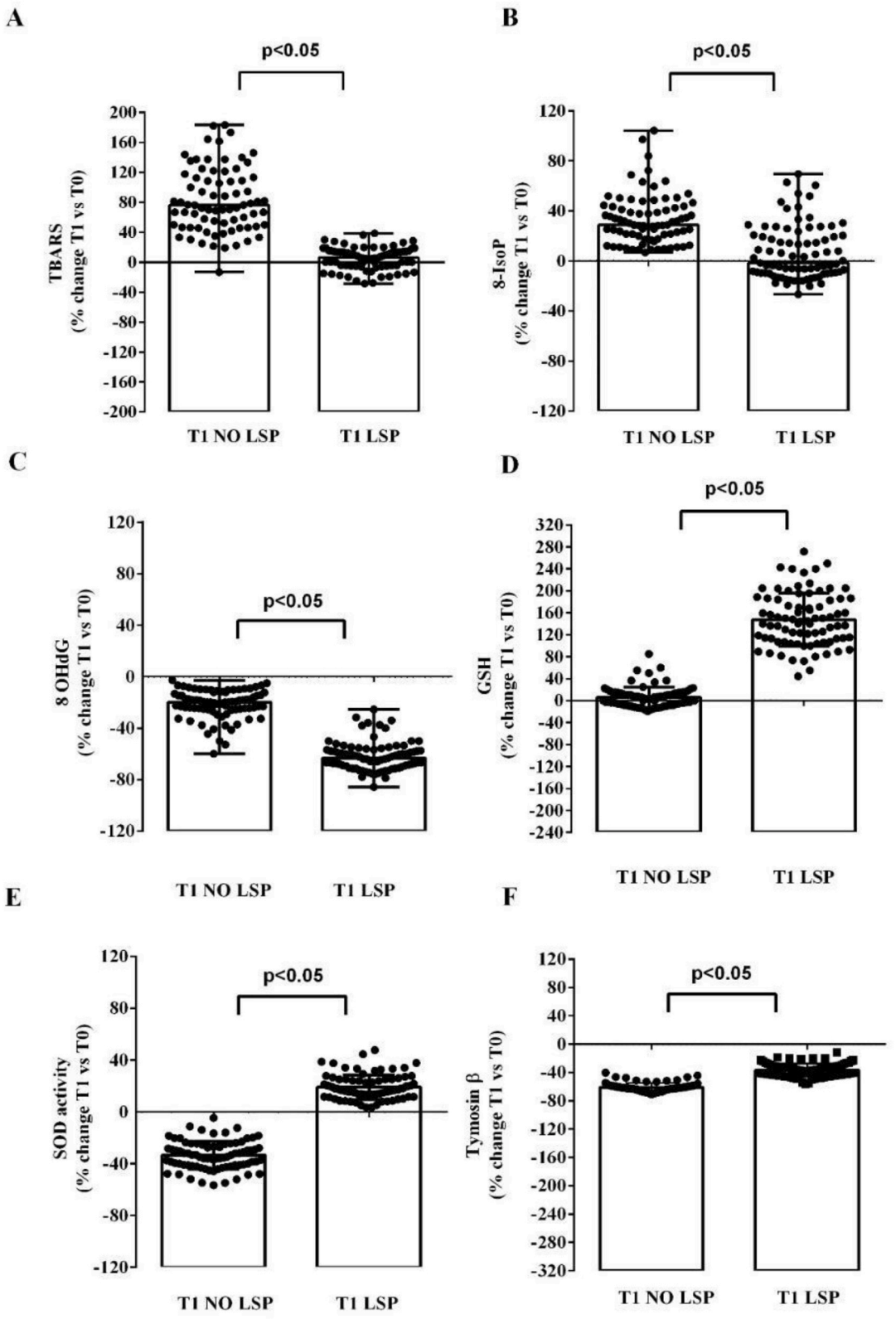

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Fig. 4. Percentage changes of plasma TBARS (A), IsoP (B), 8OHdG (C), GSH (D), SOD activity (E) and Tymosin β (F), between T1 and T0, in older people subjected to LSP (LSP) versus older people not subjected to LSP (No LSP). The results are expressed as median and range of three different measurements. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

Table 3
Baseline (T0) and T1 values of cell viability, mitochondrial membrane potential, MitoROS and ROS release of HUVEC treated with plasma of LSP group and control group.

	LSP T0	No LSP T0	LSP T1	No LSP T1	p-value
Cell viability % change vs C	39 (21–50)	40 (26–50)	41 (26–53)	29 (20–43)*	<0.05 *T1 No LSP vs T1 LSP
Mitochondrial membrane potential % change vs C	69 (50–87)	71 (48–86)	76 (60–93)	66 (42–87)*	<0.05 *T1 No LSP vs T1 LSP
MitoROS % change vs C	110 (100–120)	109 (102–121)	93 (78–103)	3 (2–3.1)*	<0.05 *T1 No LSP vs T1 LSP
ROS % change vs C	166 (140–198)	160 (131–214)	125 (107–150)	154 (118–220)*	<0.05 *T1 No LSP vs T1 LSP

MitoROS = mitochondrial ROS; ROS = reactive oxygen species; No LSP = older people not subjected to LSP. LSP = healthy life-style program. C = untreated cells. The results are expressed as median and range of experiments performed in triplicate and repeated at least three times.

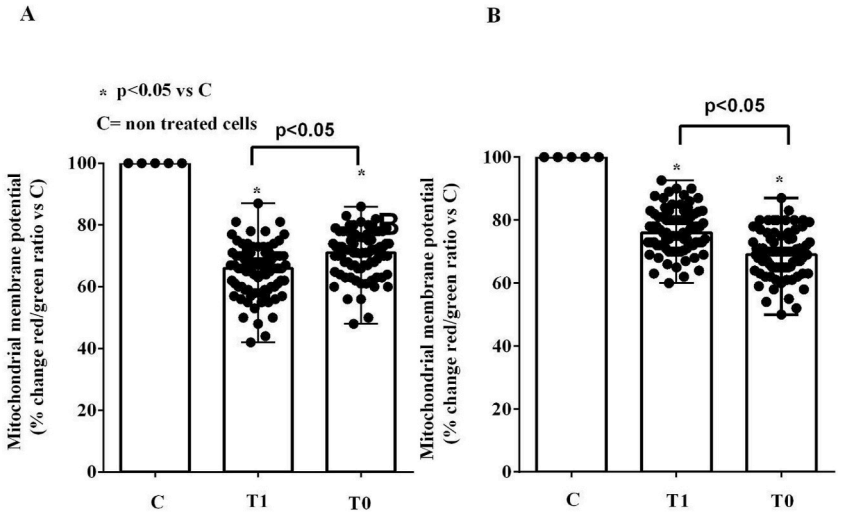

Fig. 5. Effects of plasma of older people not subjected to LSP (A) and subjected to LSP (B) on mitochondrial membrane potential of HUVEC. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

On the basis of the above considerations and on the ground of results of our previous study performed in older people who undergone a short timeframe LSP, here, we aimed to examine plasma markers of oxidative stress in those subjects, before and after the 3-months period LSP. As also above specified, we focused on oxidative stress, since it is well known to represent one of the pathogenic mechanism of the aging process, cognitive decline and aging-related pathologies [68,69]. Also, ROS release not counterbalanced by the antioxidants can induce cumulative damage to cellular components such as proteins, lipids, and DNA [70,71], which would, finally, hesitate in the onset of impaired functional phenotype.

In addition, here we analyzed the effects of plasma on mitochondrial function of endothelial cells since its impairment can contribute to the increase in oxidative stress and to the development of immune system dysfunction which is associated with unhealthy aging, the onset of chronic diseases and a greater frailty and slower gait speeds amongst patients aged ≥60 years [23,72,73].

It should be noted that in this study the oxidant and antioxidant markers, which are widely considered as mostly related to aging and aging-associated conditions, were examined [43,74].

The results we obtained showed that plasma lipid markers of peroxidation, which plays an important role in various diseases and aging processes, were increased in the subjects who had not followed LSP, only. As regarding TBARS, their measurement has been established as a global estimation of the sample lipid peroxidation level which is significantly increased in over-50-years old subjects [75–77]. It is also to note that combined training program including exercise protocols lasting more than 4 weeks was reported to dampen lipid peroxidation evaluated through TBARS as well, in elders, regardless of their intake of antioxidant supplementation [74].

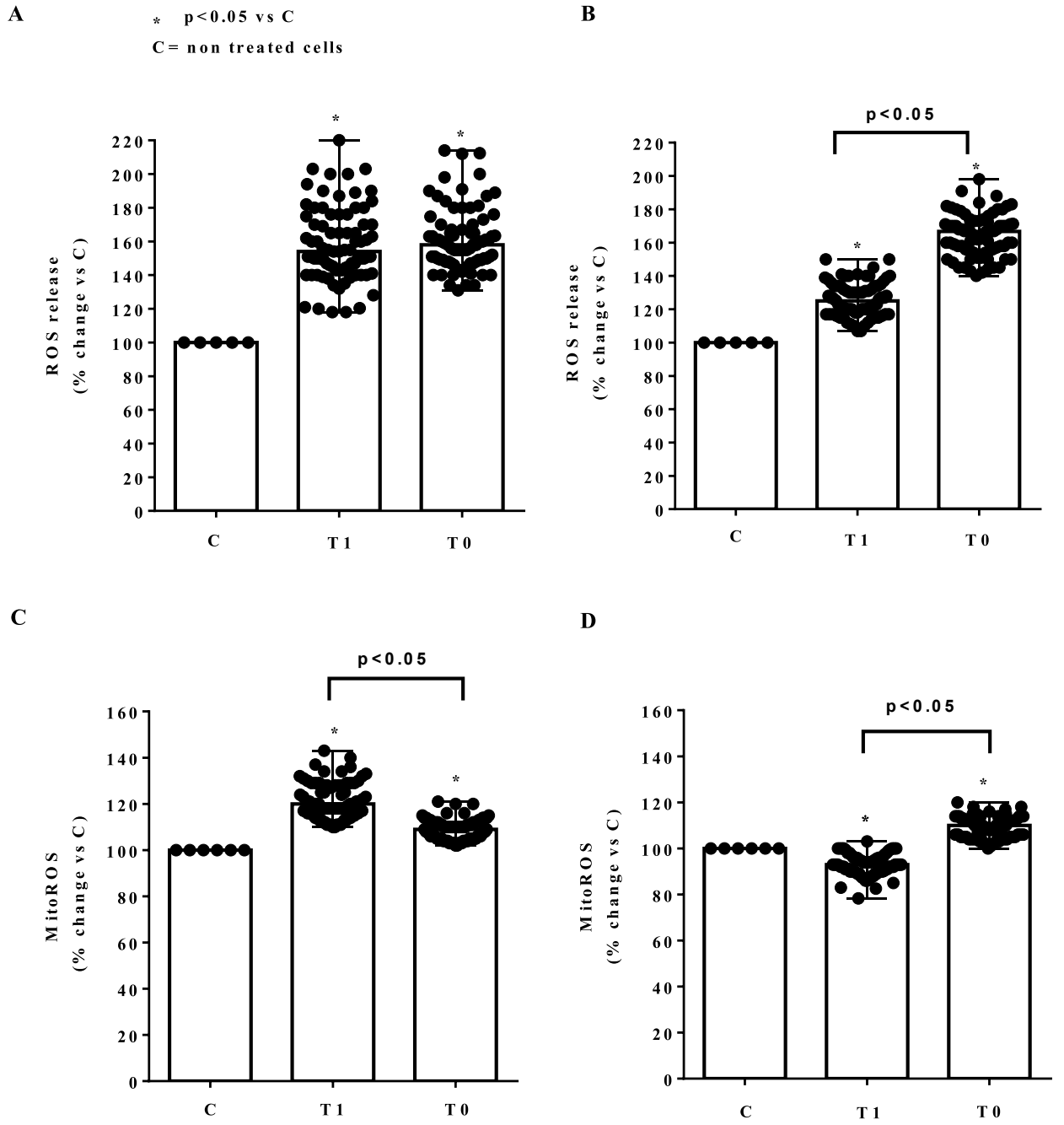

Fig. 6. Effects of plasma of older people not subjected to LSP (A) and subjected to LSP (B) on reactive oxygen species (ROS; A, B) release and mitochondrial ROS (mitoROS; C, D) release in HUVEC. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

As regarding IsoP. which belongs to a class of oxidation products arising from the peroxidation of polyunsaturated fatty acids, they are considered as accurate markers to assess OS *in vivo* and can be correlated with numerous diseases [78–80], as well as, aging [78, 80].

Furthermore, although plasma levels of 8OHdG were reduced in both groups of subjects at T1 vs T0, this reduction was more marked in LSP group. With increasing age, the risk of mitochondrial oxidative damage induced by exposure to radiation, drugs, alcohol, increases, as shown by the augmentation of the levels of 8OHdG. For this reason, 8OHdG is considered as a marker of aging and of chronic diseases [81,82]. The differences we obtained in the two groups of patients about 8OHdG could be related to the LSP, and in particular to the lower caloric intake of LSP group. Hence, caloric restriction could act as a powerful mean of slowing aging and

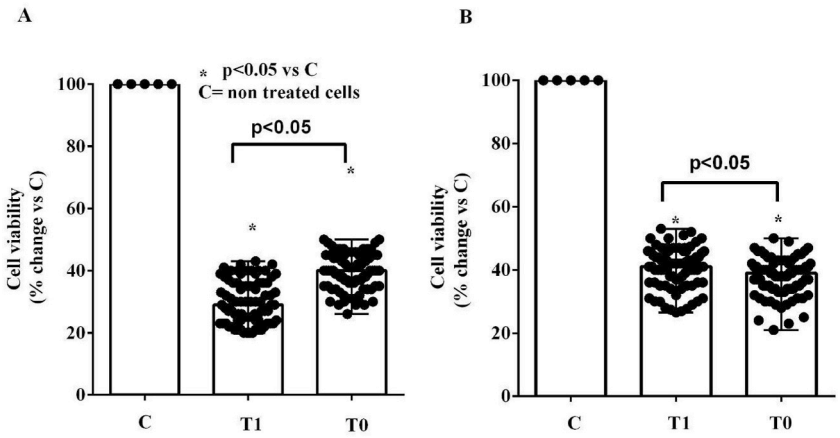

Fig. 7. Effects of plasma of older people not subjected to LSP (A) and subjected to LSP (B) on viability of HUVEC. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

increasing longevity through its antioxidants effects on damaged molecules including oxidized DNA [83].

As regarding the antioxidants, we found that in LSP group, plasma GSH was increased, whereas it did not change in the control group. Instead, the SOD activity was increased in the latter and decreased in those who did not follow the LSP.

The observed differences in the plasma levels of antioxidants, with particular reference to the SOD activity, could, therefore, explain the increase in oxidative stress markers found in subjects not undergone the LSP. Our data are in agreement with previous observations showing that physically active older people had antioxidant systems and lipid peroxidation levels similar to young sedentary subjects and emphasize the importance of regular physical activity to decelerate the ageing-associated impairment process [84–89].

In our study, plasma Tβ4 levels were reduced in both LSP and control groups at T1 compared to T0, which could be related to the aging process. However, interestingly, the subjects who followed the LSP had more preserved plasma levels of Tβ4 than those who did not followed the LSP. It is to note that Tβ4 is an immune-modulating protein released by thymus, which has drawn significant attention in regenerative medicine due to its properties in the prevention of inflammation and fibrosis and for counteracting the so-called immunesenescence [90]. Furthermore, Tβ4 was found to be expressed in the developing heart and to promote cardiac cell migration and survival. In addition, the intravenous injections of Tβ4 was able to induce cardiac changes, which resembled embryonic characteristics. For those reasons, Tβ4 was proposed as a good candidate molecule for reversing aging processes and accelerate organ regeneration in the human body [91]. Therefore, the data we obtained would add information about the role of Tβ4 as an antiaging molecule and would strengthen the role of LSP, and in particular, of physical exercise, in the improvement of features of immunesenescence [92].

As regarding the possible mechanisms underlying the beneficial effects of LSP on the above markers, available information is scarce and not conclusive. It could be hypothesized that the improvement in mitochondrial function could be at the basis of the improved redox state. Also, the increased antioxidants and findings about Tβ4 we observed in LSP group could be related to adaptative responses and "hormetic" effects induced by transcription of different redox sensitive factors including NF-κB, MAPK, and PGC-1α [93].

It is also to note that the results obtained as regarding oxidants/antioxidants were not accompanied by differences in LSP group and control group in relation to vitamin D levels. Also, the baseline values of those factors were similar in the two groups. These observations would corroborate the data we obtained regarding the beneficial effects of LSP, by eliminating potential biases related to lifestyle, diet treatments etc.

Also, the data we have obtained at the cellular level showed better results when the HUVEC were treated with the plasma of the subjects who had followed the LSP. Those findings could be related to the existence of unknown circulating factors, which could elicit the beneficial effects on HUVEC. About this issue, possible candidates could be represented by extracellular vesicles and micro RNAs, whose pattern has been reported to be affected by preventive life-style interventions [94,95].

In our study in LSP group, the mitochondrial membrane potential was increased. Instead, we found a decrease of this parameter in HUVEC treated with plasma of control group. In addition, in the intervention group, the keeping mitochondrial function was accompanied by greater effects on the oxidants release. In particular, only in HUVEC treated with plasma of LSP group both the ROS and mitoROS release was reduced. Instead, in HUVEC treated with plasma of control group, at T1 the release of mitoROS was increased and that of ROS was unchanged, compared to what observed at T0.

The results we obtained could represent the mechanism underlying the improvement observed in the subjects undergone LSP. Indeed, the intervention group, who was engaged in more physical activity and received more psychological support than the ones of control group, had better functional outcomes [29]. Furthermore, although both groups of subjects took less calories than proposed diets, LSP group showed a lower energy and carbohydrates intake than the control group. Thus, it could be hypothesized that those observations we found in the subjects of the intervention group could be related to the better redox balance, even at the cellular level, which could be driven by the aforementioned unknown circulating factors [96].

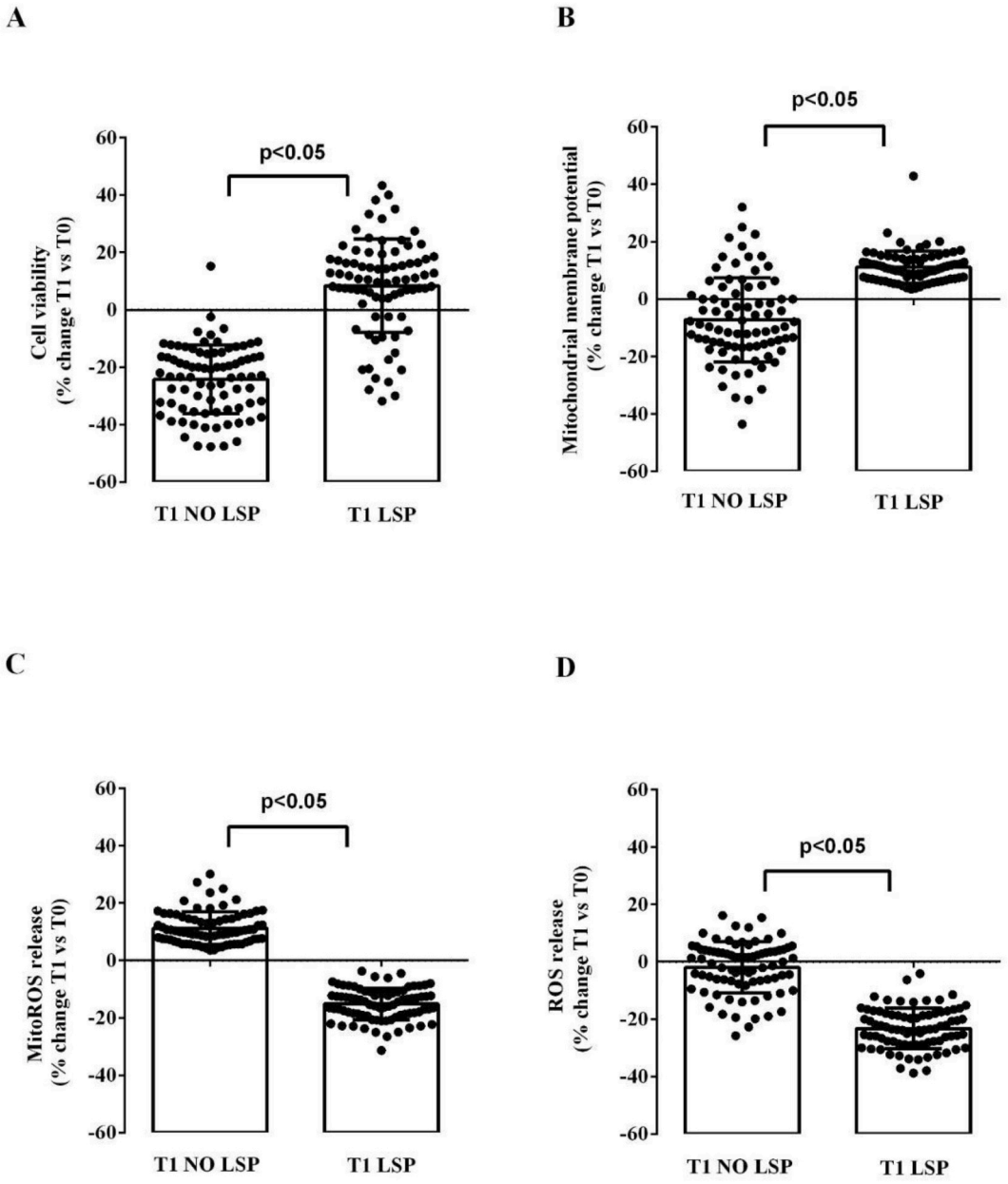

Fig. 8. Percentage changes of cell viability (A), mitochondrial membrane potential (B), mitochondrial ROS (MitoROS, C) release and ROS release (D), between T1 and T0, in HUVEC treated with plasma of older people subjected to LSP (LSP) versus older people not subjected to LSP (No LSP). The results are expressed as median and range of three different measurements. Square brackets indicate significance between groups. T0 = before LSP. T1 = after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

Also, the keeping mitochondrial function and the reduction of mitoROS release we observed in HUVEC treated with plasma of subjects undergone LSP, could be related to the results obtained about the functional scores. Indeed, an improvement in endothelial function and in particular of the mitochondria processes, could be accompanied by the maintenance of adequate tissue perfusion at the skeletal muscle, heart and central nervous system, with beneficial effects in terms of outcome and on the evaluation scales of psychophysical function.

It is to note that in this study, we focused *in vitro* on HUVEC, which are widely used vascular endothelial cells, since vascular aging, that is major risk factor for cardiovascular disease, contributes to morbidity and mortality in the older people [97]. In addition, emerging evidence shows that approaches that suppress or eliminate cellular senescence would aimed at preserving vascular function [93,98]. About this issue, it is well known that, in particular, mitochondrial dysfunction can contribute to the development of vascular aging and of all common age-related diseases [35,99]. For this reason, enhancing mitochondrial biogenesis, reducing oxidative stress

and recovering dynamics have been suggested as effective interventions to delay vascular aging and age-related cardiovascular diseases [97]. Among various possible effective antiaging strategies, the physical activity and the lowering of caloric restriction could represent possible tools, since they have been shown to elicit healthy beneficial effects in the old people when many physiological functions are in decline, through the increase of the antioxidant system, the keeping the mitochondrial function and the reduction of ROS release [30,31,34,35,37,93,100,101]. By this way, physical exercise and caloric restriction would prevent endothelial cell senescence, which can be observed with sedentary aging, and that is associated with impaired vascular endothelial function [100]. Data we obtained in HUVEC would confirm the above issues and would highlight the role of unknown circulating factors as possible mediators of the protective effects elicited by LSP. In particular, LSP would modify the pattern of those unknown circulating markers capable of inducing endothelial damage in aging, as it was already previously evidenced [43].

4.1. Study limitations

Although the data we obtained may represent innovative aspects, more information could be collected by increasing the sample size and the duration of LSP. Also, a more detailed analysis of circulating markers and of their effects on cellular types other than the endothelial ones could increase knowledge about the mechanisms at the basis of aging and of the protection exerted by healthy lifestyle programs. In addition, the nature of the unknown circulating factors should be investigated and their role in the aging and in the beneficial effects exerted by LSP better addressed.

5. Conclusion

To conclude, our study has provided new information about the pathogenic mechanisms underlying the antiaging effects of a short LSP in a group of older people living in LTC facility. In particular, our data demonstrate that a short period of LSP can improve the redox state through the increase of antioxidants, like GSH and SOD activity and the reduction of oxidants, like TBARS, 8OHdG and IsoP. At a cellular level, the LSP can improve the endothelial function though the modulation of mitochondrial efficiency. These findings could represent the mechanism underlying the improvement in the observed functional outcome of the LSP group.

Moreover, our data open the way towards further research aimed at better characterizing the nature of the above reported circulating markers. The associated implications of those findings could be related to both prognostic and therapeutic strategies, which could be pursued as antiaging methods. Hence, new tools are needed to screen the older people, to reduce frailty and to slow the progression of age-related pathophysiological changes, which lead to functional impairment.

Ethical approvals

The study was approved by the Ethical Committee of the “Azienda Ospedaliera Maggiore della Carità” University Hospital in Novara (registration number: CE 232/20) on September 30th, 2020 and complies with the Declaration of Helsinki and principles of Good Clinical Practice.

Patient consent statement

All the subjects had given their written consent to participate in the study.

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Sponsor’s role

The present study was not sponsored. The institutional funder provider (MUR; European Union-Next Generation EU) did not have any role nor influence on the study

Data availability statement

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Elena Grossini: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Sakthipryian Venkatesan:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mohammad Mostafa Ola Pour:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Andrea Conti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Conceptualization. **Diego Concina:** Writing – review & editing, Writing –

original draft, Visualization, Validation, Software, Investigation, Conceptualization. **Annalisa Opizzi:** Writing – original draft, Visualization, Validation, Software, Formal analysis. **Agatino Sanguedolce:** Writing – review & editing, Visualization, Validation, Software, Formal analysis, Data curation. **Carmela Rinaldi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Sophia Russotto:** Writing – review & editing, Visualization, Validation, Software, Formal analysis, Data curation. **Carla Maria Gramaglia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Patrizia Zeppegno:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Massimiliano Panella:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J. Guo, X. Huang, L. Dou, M. Yan, T. Shen, W. Tang, J. Li, Aging and aging-related diseases: from molecular mechanisms to interventions and treatments, Signal Transduct. Targeted Ther. 7 (2022) 391, https://doi.org/10.1038/s41392-022-01251-0.
[2] E. Grossini, D. Concina, C. Rinaldi, S. Russotto, D. Garhwal, P. Zeppegno, C. Gramaglia, S. Kul, M. Panella, Association between plasma redox state/mitochondria function and a flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit, Front. Physiol. 12 (2021) 707587, https://doi.org/10.3389/fphys.2021.707587.
[3] H.J. Shields, A. Traa, J.M. Van Raamsdonk, Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies, Front. Cell Dev. Biol. 9 (2021) 628157, https://doi.org/10.3389/fcell.2021.628157.
[4] K. Takaya, T. Asou, K. Kishi, Cistanche deserticola polysaccharide reduces inflammation and aging phenotypes in the dermal fibroblasts through the activation of the NRF2/HO-1 pathway, Int. J. Mol. Sci. 24 (2023) 15704, https://doi.org/10.3390/ijms242115704.
[5] R.A. Merchant, J.E. Morley, M. Izquierdo, Exercise, aging and frailty: guidelines for increasing function, J. Nutr. Health Aging 25 (2021) 405–409, https://doi.org/10.1007/s12603-021-1590-x.
[6] C. Cochet, G. Belloni, I. Buondonno, F. Chiara, P. D'Amelio, The role of nutrition in the treatment of sarcopenia in old patients: from restoration of mitochondrial activity to improvement of muscle performance, a systematic review, Nutrients 15 (2023) 3703, https://doi.org/10.3390/nu15173703.
[7] E. Grossini, D. Concina, C. Rinaldi, S. Russotto, D. Garhwal, P. Zeppegno, C. Gramaglia, S. Kul, M. Panella, Association between plasma redox state/mitochondria function and a flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit, Front. Physiol. 12 (2021) 707587, https://doi.org/10.3389/fphys.2021.707587.
[8] C. Ni, Y. Ji, K. Hu, K. Xing, Y. Xu, Y. Gao, Effect of exercise and antioxidant supplementation on cellular lipid peroxidation in elderly individuals: systematic review and network meta-analysis, Front. Physiol. 14 (2023) 1113270, https://doi.org/10.3389/fphys.2023.1113270.
[9] K.B. Pandey, M.M. Mehdi, P.K. Maurya, S.I. Rizvi, Plasma protein oxidation and its correlation with antioxidant potential during human aging, Dis. Markers 29 (2010) 31–36, https://doi.org/10.1155/2010/964630.
[10] S. Wu, X. Jiang, Y. Liu, L. Chen, J. Tao, Effects and mechanisms of a new multivitamin on chronic metabolic syndromes and aging, Afr. J. Tradit., Complementary Altern. Med. 14 (2016) 52–61, https://doi.org/10.21010/ajtcam.v14i1.7.
[11] T. De Carvalho, Calorie restriction or dietary restriction: how far they can protect the brain against neurodegenerative diseases? Neural Regen. Res. 17 (2022) 1640, https://doi.org/10.4103/1673-5374.332126.
[12] G.C. Román, R.E. Jackson, R. Gadhia, A.N. Román, J. Reis, Mediterranean diet: the role of long-chain ω-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and alzheimer disease, Rev. Neurol. (Paris) 175 (2019) 724–741, https://doi.org/10.1016/j.neurol.2019.08.005.
[13] A. De La Rosa, G. Olaso-Gonzalez, C. Arc-Chagnaud, F. Millan, A. Salvador-Pascual, C. García-Lucerga, C. Blasco-Lafarga, E. Garcia-Dominguez, A. Carretero, A.G. Correas, et al., Physical exercise in the prevention and treatment of alzheimer's disease, J. Sport Health Sci. 9 (2020) 394–404, https://doi.org/10.1016/j.jshs.2020.01.004.
[14] E. Anderson, J.L. Durstine, Physical activity, exercise, and chronic diseases: a brief review, Sports Med. Health Sci. 1 (2019) 3–10, https://doi.org/10.1016/j.smhs.2019.08.006.
[15] N. Fukushima, H. Kikuchi, H. Sato, H. Sasai, K. Kiyohara, S.S. Sawada, M. Machida, S. Amagasa, S. Inoue, Dose-response relationship of physical activity with all-cause mortality among older adults: an umbrella review, J. Am. Med. Dir. Assoc. 25 (2024) 417–430, https://doi.org/10.1016/j.jamda.2023.09.028.
[16] M. Wicks, A.M. Dennett, C.L. Peiris, Physiotherapist-led, exercise-based telerehabilitation for older adults improves patient and health service outcomes: a systematic review and meta-analysis, Age Ageing 52 (2023) afad207, https://doi.org/10.1093/ageing/afad207.
[17] Y.-H. Lin, Y.-C. Chen, Y.-C. Tseng, S. Tsai, Y.-H. Tseng, Physical activity and successful aging among middle-aged and older adults: a systematic review and meta-analysis of cohort studies, Aging 12 (2020) 7704–7716, https://doi.org/10.18632/aging.103057.
[18] K.L. Moreau, Z.S. Clayton, L.E. DuBose, R. Rosenberry, D.R. Seals, Effects of regular exercise on vascular function with aging: does sex matter? Am. J. Physiol. Heart Circ. Physiol. 326 (2024) H123–H137, https://doi.org/10.1152/ajpheart.00392.2023.
[19] B. Langhammer, A. Bergland, E. Rydwik, The importance of physical activity exercise among older people, BioMed Res. Int. 2018 (2018) 1–3, https://doi.org/10.1155/2018/7856823.
[20] T. Govindaraju, B. Sahle, T. McCaffrey, J. McNeil, A. Owen, Dietary patterns and quality of life in older adults: a systematic review, Nutrients 10 (2018) 971, https://doi.org/10.3390/nu10080971.
[21] L.K. English, J.D. Ard, R.L. Bailey, M. Bates, L.A. Bazzano, C.J. Boushey, C. Brown, G. Butera, E.H. Callahan, J. De Jesus, et al., Evaluation of dietary patterns and all-cause mortality: a systematic review, JAMA Netw. Open 4 (2021) e2122277, https://doi.org/10.1001/jamanetworkopen.2021.22277.
[22] R. Militello, S. Luti, T. Gamberi, A. Pellegrino, A. Modesti, P.A. Modesti, Physical activity and oxidative stress in aging, Antioxidants 13 (2024) 557, https://doi.org/10.3390/antiox13050557.

[23] E. Maldonado, S. Morales-Pison, F. Urbina, A. Solari, Aging hallmarks and the role of oxidative stress, Antioxidants 12 (2023) 651, https://doi.org/10.3390/antiox12030651.

[24] T. Gamberi, Effect of functional fitness on plasma oxidation level in elders: reduction of the plasma oxidants and improvement of the antioxidant barrier, Am. J. Sports Sci. 6 (2018) 55, https://doi.org/10.11648/j.ajss.20180602.14.

[25] B.A. Muzembo, D. Narongpon, N.R. Ngatu, M. Eitoku, R. Hirota, N. Suganuma, Assessment of lifestyle effect on oxidative stress biomarkers in free-living elderly in rural Japan, Geriatr. Gerontol. Int. 12 (2012) 547–554, https://doi.org/10.1111/j.1447-0594.2011.00793.x.

[26] M.V. Bacanoiu, M. Danoiu, L. Rusu, M.I. Marin, New directions to approach oxidative stress related to physical activity and nutraceuticals in normal aging and neurodegenerative aging, Antioxidants 12 (2023) 1008, https://doi.org/10.3390/antiox12051008.

[27] S. Husain, K. Hillmann, K. Hengst, H. Englert, Effects of a lifestyle intervention on the biomarkers of oxidative stress in non-communicable diseases: a systematic review, Front. Aging 4 (2023), https://doi.org/10.3389/fragi.2023.1085511.

[28] M. Baierle, S.N. Nascimento, A.M. Moro, N. Brucker, F. Freitas, B. Gauer, J. Durgante, S. Bordignon, M. Zibetti, C.M. Trentini, et al., Relationship between inflammation and oxidative stress and cognitive decline in the institutionalized elderly, Oxid. Med. Cell. Longev. 2015 (2015) 804198, https://doi.org/10.1155/2015/804198.

[29] A. Conti, D. Concina, A. Opizzi, A. Sanguedolce, C. Rinaldi, S. Russotto, E. Grossini, C.M. Gramaglia, P. Zeppegno, M. Panella, Effectiveness of a combined lifestyle intervention for older people in long-term care: a randomized controlled trial, Arch. Gerontol. Geriatr. 120 (2024) 105340, https://doi.org/10.1016/j.archger.2024.105340.

[30] M. Nakahama-Matsushima, Y. Kamijyo, Y. Umemoto, T. Hashizaki, Y. Nishimura, K. Furusawa, Y. Furotani, F. Tajima, K. Kouda, Increase in serum interleukin-1 receptor antagonist (IL-1ra) levels after wheelchair half marathon race in male athletes with spinal cord injury, J. Clin. Med. 12 (2023) 7098, https://doi.org/10.3390/jcm12227098.

[31] J. Angulo, M. El Assar, A. Álvarez-Bustos, L. Rodríguez-Mañas, Physical activity and exercise: strategies to manage frailty, Redox Biol. 35 (2020) 101513, https://doi.org/10.1016/j.redox.2020.101513.

[32] D.K. Das, Z.A. Graham, C.P. Cardozo, Myokines in skeletal muscle Physiology and metabolism: recent advances and future perspectives, Acta Physiol. 228 (2020) e13367, https://doi.org/10.1111/apha.13367.

[33] M. El Assar, A. Álvarez-Bustos, P. Sosa, J. Angulo, L. Rodríguez-Mañas, Effect of physical activity/exercise on oxidative stress and inflammation in muscle and vascular aging, Int. J. Mol. Sci. 23 (2022) 8713, https://doi.org/10.3390/ijms23158713.

[34] S.F. Vatner, J. Zhang, M. Oydanich, T. Berkman, R. Naftalovich, D.E. Vatner, Healthful aging mediated by inhibition of oxidative stress, Ageing Res. Rev. 64 (2020) 101194, https://doi.org/10.1016/j.arr.2020.101194.

[35] K. Jomova, R. Raptova, S.Y. Alomar, S.H. Alwasel, E. Nepovimova, K. Kuca, M. Valko, Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging, Arch. Toxicol. 97 (2023) 2499–2574, https://doi.org/10.1007/s00204-023-03562-9.

[36] S. Bayod, J. Del Valle, J.F. Lalanza, S. Sanchez-Roige, B. de Luxán-Delgado, A. Coto-Montes, A.M. Canudas, A. Camins, R.M. Escorihuela, M. Pallàs, Long-term physical exercise induces changes in sirtuin 1 pathway and oxidative parameters in adult rat tissues, Exp. Gerontol. 47 (2012) 925–935, https://doi.org/10.1016/j.exger.2012.08.004.

[37] A. Ionescu-Tucker, C.W. Cotman, Emerging roles of oxidative stress in brain aging and alzheimer's disease, Neurobiol. Aging 107 (2021) 86–95, https://doi.org/10.1016/j.neurobiolaging.2021.07.014.

[38] L. Roth, S. Dogan, B.G. Tuna, T. Aranyi, S. Benitez, M. Borrell-Pages, P. Bozaykut, G.R.Y. De Meyer, L. Duca, N. Durmus, et al., Pharmacological modulation of vascular ageing: a review from VascAgeNet, Ageing Res. Rev. 92 (2023) 102122, https://doi.org/10.1016/j.arr.2023.102122.

[39] E. Belotti, N. Lacoste, A. Iftikhar, T. Simonet, C. Papin, A. Osseni, N. Streichenberger, P.-O. Mari, E. Girard, M. Graies, et al., H2A.Z is involved in premature aging and DSB repair initiation in muscle fibers, Nucleic Acids Res. 52 (2024) 3031–3049, https://doi.org/10.1093/nar/gkae020.

[40] X. Gao, X. Yu, C. Zhang, Y. Wang, Y. Sun, H. Sun, H. Zhang, Y. Shi, X. He, Telomeres and mitochondrial metabolism: implications for cellular senescence and age-related diseases, Stem Cell Rev. Rep. 18 (2022) 2315–2327, https://doi.org/10.1007/s12015-022-10370-8.

[41] A. Koller, C. Lamina, C. Brandl, M.E. Zimmermann, K.J. Stark, H. Weissensteiner, R. Würzner, I.M. Heid, F. Kronenberg, Systemic evidence for mitochondrial dysfunction in age-related macular degeneration as revealed by mtDNA copy number measurements in peripheral blood, Int. J. Mol. Sci. 24 (2023) 16406, https://doi.org/10.3390/ijms242216406.

[42] A. Varesi, S. Chirumbolo, L.I.M. Campagnoli, E. Pierella, G.B. Piccini, A. Carrara, G. Ricevuti, C. Scassellati, C. Bonvicini, A. Pascale, The role of antioxidants in the interplay between oxidative stress and senescence, Antioxidants 11 (2022) 1224, https://doi.org/10.3390/antiox11071224.

[43] E. Grossini, D. Concina, C. Rinaldi, S. Russotto, D. Garhwal, P. Zeppegno, C. Gramaglia, S. Kul, M. Panella, Association between plasma redox state/mitochondria function and a flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit, Front. Physiol. 12 (2021) 707587, https://doi.org/10.3389/fphys.2021.707587.

[44] R. Ramírez, N. Ceprian, A. Figuer, G. Valera, G. Bodega, M. Alique, J. Carracedo, Endothelial senescence and the chronic vascular diseases: challenges and therapeutic opportunities in atherosclerosis, J. Personalized Med. 12 (2022) 215, https://doi.org/10.3390/jpm12020215.

[45] A. Turnbull, A. Seitz, D. Tadin, F.V. Lin, Unifying framework for cognitive training interventions in brain aging, Ageing Res. Rev. 81 (2022) 101724, https://doi.org/10.1016/j.arr.2022.101724.

[46] F. Mahoney, Functional evaluation: the Barthel Index, Md. State Med. J. 14 (1965) 61–65.

[47] S. Katz, Studies of illness in the aged: the Index of ADL: a standardized measure of biological and psychosocial function, JAMA 185 (1963) 914, https://doi.org/10.1001/jama.1963.03060120024016.

[48] M.E. Tinetti, Performance-oriented assessment of mobility problems in elderly patients, J. Am. Geriatr. Soc. 34 (1986) 119–126, https://doi.org/10.1111/j.1532-5415.1986.tb05480.x.

[49] I.-T. Liu, W.-J. Lee, S.-Y. Lin, S.-T. Chang, C.-L. Kao, Y.-Y. Cheng, Therapeutic effects of exercise training on elderly patients with dementia: a randomized controlled trial, Arch. Phys. Med. Rehabil. 101 (2020) 762–769, https://doi.org/10.1016/j.apmr.2020.01.012.

[50] K. Walters, R. Frost, K. Kharicha, C. Avgerinou, B. Gardner, F. Ricciardi, R. Hunter, A. Liljas, J. Manthorpe, V. Drennan, et al., Home-based health promotion for older people with mild frailty: the HomeHealth intervention development and feasibility RCT, Health Technol. Assess. 21 (2017) 1–128, https://doi.org/10.3310/hta21730.

[51] S. Damanti, D. Azzolino, C. Roncaglione, B. Arosio, P. Rossi, M. Cesari, Efficacy of nutritional interventions as stand-alone or synergistic treatments with exercise for the management of sarcopenia, Nutrients 11 (2019) 1991, https://doi.org/10.3390/nu11091991.

[52] B.C. Clark, T.M. Manini, Functional consequences of sarcopenia and dynapenia in the elderly, Curr. Opin. Clin. Nutr. Metab. Care 13 (2010) 271–276, https://doi.org/10.1097/MCO.0b013e328337819e.

[53] D. Verhaegen, K. Smits, N. Osório, A. Caseiro, Oxidative stress in relation to aging and exercise, Encyclopedia 2 (2022) 1545–1558, https://doi.org/10.3390/encyclopedia2030105.

[54] S. Chen, A. Calderón-Larrañaga, M. Saadeh, I.-M. Dohrn, A.-K. Welmer, Correlations of subjective and social well-being with sedentary behavior and physical activity in older adults-A population-based study, J. Gerontol. A. Biol. Sci. Med. Sci. 76 (2021) 1789–1795, https://doi.org/10.1093/gerona/glab065.

[55] A.M. Holwerda, K.J.M. Paulussen, M. Overkamp, J.P.B. Goessens, I.-F. Kramer, W.K.W.H. Wodzig, L.B. Verdijk, L.C.P.G.M. De Groot, L.J.C. Van Loon, Leucine coingestion augments the muscle protein synthetic response to the ingestion of 15 g of protein following resistance exercise in older men, Am. J. Physiol. Endocrinol. Metab. 317 (2019) E473–E482, https://doi.org/10.1152/ajpendo.00073.2019.

[56] Z. Hamid, A. Riggs, T. Spencer, C. Redman, D. Bodenner, Vitamin D deficiency in residents of academic long-term care facilities despite having been prescribed vitamin D, J. Am. Med. Dir. Assoc. 8 (2007) 71–75, https://doi.org/10.1016/j.jamda.2006.07.005.

[57] Società Italiana di Nutrizione Umana Livelli Di Assunzione Di Riferimento Di Nutrienti Ed Energia per La Popolazione Italiana (LARN) Available online: https://sinu.it/tabelle-larn-2014/(accessed on 15 May 2023).

[58] M. López-Moreno, M. Garcés-Rimón, M. Miguel, Antinutrients: lectins, goitrogens, phytates and oxalates, friends or foe? J. Funct.Foods 89 (2022) 104938 https://doi.org/10.1016/j.jff.2022.104938.

[59] E. Grossini, D. Garhwal, S. Venkatesan, D. Ferrante, A. Mele, M. Saraceno, A. Scognamiglio, J. Mandrioli, A. Amedei, F. De Marchi, et al., The potential role of peripheral oxidative stress on the neurovascular unit in amyotrophic lateral sclerosis pathogenesis: a preliminary report from human and in vitro evaluations, Biomedicines 10 (2022) 691, https://doi.org/10.3390/biomedicines10030691.

[60] E. Grossini, S. Farruggio, D. Pierelli, V. Bolzani, L. Rossi, P. Pollesello, C. Monaco, Levosimendan improves oxidative balance in cardiogenic shock/low cardiac output patients, J. Clin. Med. 9 (2020) 373, https://doi.org/10.3390/jcm9020373.

[61] E. Grossini, F. De Marchi, S. Venkatesan, A. Mele, D. Ferrante, L. Mazzini, Effects of acetyl-L-carnitine on oxidative stress in amyotrophic lateral sclerosis patients: evaluation on plasma markers and members of the neurovascular unit, Antioxidants 12 (2023) 1887, https://doi.org/10.3390/antiox12101887.

[62] E. Grossini, C. Smirne, S. Venkatesan, S. Tonello, D. D'Onghia, R. Minisini, V. Cantaluppi, P.P. Sainaghi, C. Comi, A. Tanzi, et al., Plasma pattern of extracellular vesicles isolated from hepatitis C virus patients and their effects on human vascular endothelial cells, Int. J. Mol. Sci. 24 (2023) 10197, https://doi.org/10.3390/ijms241210197.

[63] E. Grossini, S. Venkatesan, M. Alkabes, C. Toma, S. De Cillà, Membrane blue dual protects retinal pigment epithelium cells/ganglion cells—like through modulation of mitochondria function, Biomedicines 10 (2022) 2854, https://doi.org/10.3390/biomedicines10112854.

[64] E. Grossini, T. Esposito, M. Viretto, S. Venkatesan, I. Licari, D. Surico, F. Della Corte, L. Castello, S. Bruno, M. Quaglia, et al., Circulating extracellular vesicles in subarachnoid hemorrhage patients: characterization and cellular effects, Int. J. Mol. Sci. 24 (2023) 14913, https://doi.org/10.3390/ijms241914913.

[65] N.J. Roos, U. Duthaler, J. Bouitbir, S. Krähenbühl, The uricosuric benzbromarone disturbs the mitochondrial redox homeostasis and activates the NRF2 signaling pathway in HepG2 cells, Free Radic. Biol. Med. 152 (2020) 216–226, https://doi.org/10.1016/j.freeradbiomed.2020.03.009.

[66] M. Bouzid, E. Filaire, R. Matran, S. Robin, C. Fabre, Lifelong voluntary exercise modulates age-related changes in oxidative stress, Int. J. Sports Med. 39 (2018) 21–28, https://doi.org/10.1055/s-0043-119882.

[67] A. Santoro, M. Martucci, M. Conte, M. Capri, C. Franceschi, S. Inflammaging Salvioli, Hormesis and the rationale for anti-aging strategies, Ageing Res. Rev. 64 (2020) 101142, https://doi.org/10.1016/j.arr.2020.101142.

[68] C. Rojas-Solé, V. Pinilla-González, J. Lillo-Moya, T. González-Fernández, L. Saso, R. Rodrigo, Integrated approach to reducing polypharmacy in older people: exploring the role of oxidative stress and antioxidant potential therapy, Redox Rep. 29 (2024) 2289740, https://doi.org/10.1080/13510002.2023.2289740.

[69] C. Vinel, O. Pereira, A. Dupuy, J. Bertrand-Michel, D. Laoudj-Chenivesse, Y. Rolland, D. Rivière, P. Valet, C. Dray, F. Pillard, Isoprostanes as markers for muscle aging in older athletes, Biochim. Open 6 (2018) 1–8, https://doi.org/10.1016/j.biopen.2017.12.001.

[70] M.B. Kadiiska, B.C. Gladen, D.D. Baird, D. Germolec, L.B. Graham, C.E. Parker, A. Nyska, J.T. Wachsman, B.N. Ames, S. Basu, et al., Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic. Biol. Med. 38 (2005) 698–710, https://doi.org/10.1016/j.freeradbiomed.2004.09.017.

[71] P. Montuschi, P.J. Barnes, L.J. Roberts, Isoprostanes: markers and mediators of oxidative stress, FASEB J. 18 (2004) 1791–1800, https://doi.org/10.1096/fj.04-2330rev.

[72] C.K. Liu, A. Lyass, M.G. Larson, J.M. Massaro, N. Wang, R.B. D'Agostino, E.J. Benjamin, J.M. Murabito, Biomarkers of oxidative stress are associated with frailty: the framingham offspring study, AGE 38 (2016) 1, https://doi.org/10.1007/s11357-015-9864-z.

[73] J.A. Woods, K.R. Wilund, S.A. Martin, B.M. Kistler, Exercise, inflammation and aging, Aging Dis 3 (2012) 130–140.

[74] C. Ni, Y. Ji, K. Hu, K. Xing, Y. Xu, Y. Gao, Effect of exercise and antioxidant supplementation on cellular lipid peroxidation in elderly individuals: systematic review and network meta-analysis, Front. Physiol. 14 (2023) 1113270, https://doi.org/10.3389/fphys.2023.1113270.

[75] H. Yavuzer, S. Yavuzer, M. Cengiz, H. Erman, A. Doventas, H. Balci, D.S. Erdincler, H. Uzun, Biomarkers of lipid peroxidation related to hypertension in aging, Hypertens. Res. 39 (2016) 342–348, https://doi.org/10.1038/hr.2015.156.

[76] N. Vazquez-Agra, A.-T. Marques-Afonso, A. Cruces-Sande, I. Novo-Veleiro, A. Pose-Reino, E. Mendez-Alvarez, R. Soto-Otero, A. Hermida-Ameijeiras, Assessment of oxidative stress markers in elderly patients with SARS-CoV-2 infection and potential prognostic implications in the medium and long term, PLoS One 17 (2022) e0268871, https://doi.org/10.1371/journal.pone.0268871.

[77] S.S. Chan, H.P. Monteiro, G.P. Deucher, R.L. Abud, D. Abuchalla, V.B. Junqueira, Functional activity of blood polymorphonuclear leukocytes as an oxidative stress biomarker in human subjects, Free Radic. Biol. Med. 24 (1998) 1411–1418, https://doi.org/10.1016/s0891-5849(98)00010-0.

[78] J. Guest, R. Grant, T.A. Mori, K.D. Croft, Changes in oxidative damage, inflammation and [NAD(H)] with age in cerebrospinal fluid, PLoS One 9 (2014) e85335, https://doi.org/10.1371/journal.pone.0085335.

[79] D.A. Camfield, K. Nolidin, K. Savage, J. Timmer, K. Croft, M. Tangestani Fard, T. Simpson, L. Downey, A. Scholey, A. Pipingas, et al., Higher plasma levels of F_2 -isoprostanes are associated with slower psychomotor speed in healthy older adults, Free Radic. Res. 53 (2019) 377–386, https://doi.org/10.1080/10715762.2018.1513133.

[80] T.J. Montine, E.R. Peskind, J.F. Quinn, A.M. Wilson, K.S. Montine, D. Galasko, Increased cerebrospinal fluid F2-isoprostanes are associated with aging and latent alzheimer's disease as identified by biomarkers, NeuroMolecular Med. 13 (2011) 37–43, https://doi.org/10.1007/s12017-010-8126-6.

[81] Y. Zhao, W. Liang, S. Tian, L. Shen, H. Yang, Impact of increased serum 8-hydroxy-2′-deoxyguanosine levels on extent of coronary artery lesions in elderly patients with type 2 diabetes, J. Int. Med. Res. 48 (2020) 030006052093465, https://doi.org/10.1177/0300060520934653.

[82] K. Syslová, A. Böhmová, M. Mikoška, M. Kuzma, D. Pelclová, P. Kačer, Multimarker screening of oxidative stress in aging, Oxid. Med. Cell. Longev. 2014 (2014) 1–14, https://doi.org/10.1155/2014/562860.

[83] A. Donati, G. Cavallini, E. Bergamini, Effects of aging, antiaging calorie restriction and in vivo stimulation of autophagy on the urinary excretion of 8OHdG in male sprague–dawley rats, AGE 35 (2013) 261–270, https://doi.org/10.1007/s11357-011-9346-x.

[84] R.A. Merchant, J.E. Morley, M. Izquierdo, Exercise, aging and frailty: guidelines for increasing function, J. Nutr. Health Aging 25 (2021) 405–409, https://doi.org/10.1007/s12603-021-1590-x.

[85] M. Kozakiewicz, R. Rowiński, M. Kornatowski, A. Dąbrowski, K. Kędziora-Kornatowska, A. Strachecka, Relation of moderate physical activity to blood markers of oxidative stress and antioxidant defense in the elderly, Oxid. Med. Cell. Longev. 2019 (2019) 1–7, https://doi.org/10.1155/2019/5123628.

[86] H.J. Coelho-Júnior, B. Rodrigues, Exercise and aging: different approaches to different beneficial effects, Gerontol. Geriatr. Med. 3 (2017) 233372141773519, https://doi.org/10.1177/2333721417735198.

[87] C. Simioni, G. Zauli, A.M. Martelli, M. Vitale, G. Sacchetti, A. Gonelli, L.M. Neri, Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging, Oncotarget 9 (2018) 17181–17198, https://doi.org/10.18632/oncotarget.24729.

[88] Z. Radak, F. Torma, I. Berkes, S. Goto, T. Mimura, A. Posa, L. Balogh, I. Boldogh, K. Suzuki, M. Higuchi, et al., Exercise effects on physiological function during aging, Free Radic. Biol. Med. 132 (2019) 33–41, https://doi.org/10.1016/j.freeradbiomed.2018.10.444.

[89] R. Rowiński, M. Kozakiewicz, K. Kędziora-Kornatowska, E. Hübner-Woźniak, J. Kędziora, Markers of oxidative stress and erythrocyte antioxidant enzyme activity in older men and women with differing physical activity, Exp. Gerontol. 48 (2013) 1141–1146, https://doi.org/10.1016/j.exger.2013.07.010.

[90] R. Shah, K. Reyes-Gordillo, Y. Cheng, R. Varatharajalu, J. Ibrahim, M.R. Lakshman, Thymosin β 4 prevents oxidative stress, inflammation, and fibrosis in ethanol- and LPS-induced liver injury in mice, Oxid. Med. Cell. Longev. 2018 (2018) 1–12, https://doi.org/10.1155/2018/9630175.

[91] I. Bock-Marquette, K. Maar, S. Maar, B. Lippai, G. Faskerti, F. Gallyas Jr, E.N. Olson, D. Srivastava, Thymosin beta-4 denotes new directions towards developing prosperous anti-aging regenerative therapies, Int. Immunopharmacol. 116 (2023) 109741, https://doi.org/10.1016/j.intimp.2023.109741.

[92] N.A. Duggal, R.D. Pollock, N.R. Lazarus, S. Harridge, J.M. Lord, Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood, Aging Cell 17 (2018) e12750, https://doi.org/10.1111/acel.12750.

[93] M. El Assar, A. Álvarez-Bustos, P. Sosa, J. Angulo, L. Rodríguez-Mañas, Effect of physical activity/exercise on oxidative stress and inflammation in muscle and vascular aging, Int. J. Mol. Sci. 23 (2022) 8713, https://doi.org/10.3390/ijms23158713.

[94] K. Garai, Z. Adam, R. Herczeg, K. Banfai, A. Gyebrovszki, A. Gyenesei, J.E. Pongracz, M. Wilhelm, K. Kvell, Physical activity as a preventive lifestyle intervention acts through specific exosomal miRNA species—evidence from human short- and long-term pilot studies, Front. Physiol. 12 (2021) 658218, https://doi.org/10.3389/fphys.2021.658218.

[95] K. Garai, Z. Adam, R. Herczeg, K. Banfai, A. Gyebrovszki, A. Gyenesei, J.E. Pongracz, M. Wilhelm, K. Kvell, Corrigendum: physical activity as a preventive lifestyle intervention acts through specific exosomal miRNA species—evidence from human short- and long-term pilot studies, Front. Physiol. 12 (2021) 794940, https://doi.org/10.3389/fphys.2021.794940.
[96] Y.H. Lee, M.U. Kuk, M.K. So, E.S. Song, H. Lee, S.K. Ahn, H.W. Kwon, J.T. Park, S.C. Park, Targeting mitochondrial oxidative stress as a strategy to treat aging and age-related diseases, Antioxidants 12 (2023) 934, https://doi.org/10.3390/antiox12040934.
[97] Y. Li, X. Jin, D. Li, J. Lu, X. Zhang, S. Yang, Y. Zhao, M. Wu, New insights into vascular aging: emerging role of mitochondria function, Biomed. Pharmacother. 156 (2022) 113954, https://doi.org/10.1016/j.biopha.2022.113954.
[98] J. Meng, Q. Geng, S. Jin, X. Teng, L. Xiao, Y. Wu, D. Tian, Exercise protects vascular function by countering senescent cells in older adults, Front. Physiol. 14 (2023) 1138162, https://doi.org/10.3389/fphys.2023.1138162.
[99] D.-F. Dai, P.S. Rabinovitch, Z. Ungvari, Mitochondria and cardiovascular aging, Circ. Res. 110 (2012) 1109–1124, https://doi.org/10.1161/CIRCRESAHA.111.246140.
[100] M.J. Rossman, R.E. Kaplon, S.D. Hill, M.N. McNamara, J.R. Santos-Parker, G.L. Pierce, D.R. Seals, A.J. Donato, Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function, Am. J. Physiol. Heart Circ. Physiol. 313 (2017) H890–H895, https://doi.org/10.1152/ajpheart.00416.2017.
[101] E.W. Flanagan, J. Most, J.T. Mey, L.M. Redman, Calorie restriction and aging in humans, Annu. Rev. Nutr. 40 (2020) 105–133, https://doi.org/10.1146/annurev-nutr-122319-034601.