Dolichol - a component of the cellular antioxidant machinery

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[‡]This paper is dedicated to the memory of Gabriele Chiti, a skilful technician, a dear friend of Biophysics Institute of the National Research Council prematurely passed away

Keywords: Dolichol, Coenzyme Q, α–tocopherol, Phospholipid-bound polyunsaturated fatty acids, Rat hepatocytes, Oxidative stress

Abbreviations:

CoQ	Coenzyme Q
DCFH-DA	2',7'-dichlorodihydro-fuorescein diacetate
FA	Fatty acid(s)
FAME	Fatty acid methyl esters
GC	Gas-liquid chromatography
HMGCoAR	3-hydroxy 3-methylglutaryl CoA reductase
HPLC	High-pressure liquid chromatography
MDA	Malondialdehyde
OS	Oxidative stress
PL	Phospholipid(s)
PL PUFA	Phospholipid-bound polyunsaturated fatty acid(s)
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UV-B	Ultraviolet-B

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ABSTRACT

Dolichol, an end product of the mevalonate pathway, has been proposed a biomarker of aging, but its biological role, not to mention its catabolism, has not been fully understood. UV-B radiation was used to induce oxidative stress in isolated rat hepatocytes by the collagenase method. Effects on dolichol, phospholipids-bound polyunsaturated fatty acids (PL PUFA) and known lipid soluble antioxidants [coenzyme Q (CoQ) and α-tocopherol] were studied. The increase in oxidative stress was detected by a probe sensitive to reactive oxygen species (ROS). Peroxidation of lipids was assessed by measuring the release of thiobarbituric acid reactive substances (TBARS). Dolichol, CoQ and α-tocopherol were assessed by high-pressure liquid chromatography (HPLC), PL PUFA by gas-liquid chromatography (GC). UV-B radiation caused an immediate increase in ROS as well as lipid peroxidation and a simultaneous decrease in the levels of dolichol and lipid soluble antioxidants. Decrease in dolichol paralleled changes in CoQ levels and was smaller than that in α-tocopherol. The addition of mevinolin, a competitive inhibitor of the enzyme 3-hydroxy 3-methylglutaryl CoA reductase (HMGCoAR), magnified the loss of dolichol and was associated with an increase in TBARS production. Changes in PL PUFA were minor. These findings highlight that oxidative stress has very early and similar effects on dolichol and lipid soluble antioxidants. Lower levels of dolichol are associated with enhanced peroxidation of lipids, which suggest that dolichol may have a protective role in the antioxidant machinery of cell membranes and perhaps be a key to understanding some adverse effects of statin therapy.

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Introduction

There is increasing recognition that many chronic degenerative diseases of older age, including cancer, diabetes, atherosclerosis, cardiovascular diseases, inflammatory diseases and aging itself, are due to the oxidative stress (OS) that arises due to disturbed equilibrium between pro-oxidant/antioxidant homeostasis [1, 2]. OS is initiated by free radical species which become stable through electron pairing with biological macromolecules such as proteins, lipids and DNA in cells and cause protein and DNA damage along with lipid peroxidation. Overproduction or incorporation of free radicals from environment to living system may enhance the rate of the process (extrinsic aging). All biological systems are endowed with antioxidant defense mechanisms that protect against oxidative injuries and with enzymes that remove damaged molecules [3]. The damage can become more widespread due to imbalanced or weakened cellular antioxidant defense systems.

Dolichol is a polyisoprenic molecule present in all tissues and most organelle membranes of eukaryotic cells [4]. An interesting finding was that dolichol progressively accumulates in tissues during growth [5], maturation [5, 6] and older age [7, 8]. It was shown that the age-related accumulation of dolichol in tissues meets all criteria to be qualified as a biomarker of aging [9]. Probably, the understanding of the mechanisms underlying its age-related accumulation might shed light on the metabolic lesions primarily involved in the aging process and many diseases of old age. It is noteworthy that dolichol is one end product of the mevalonate pathway, together with ubiquinone and cholesterol [10], and increasing age could be associated with specific changes in regulation of mevalonate pathway flux. Data show that the tissue dolichol accumulation is connected with the age-related constitutive activation of HMGCoAR, the rate-limiting enzyme of isoprenoid biosynthesis [11, 12], probably mediated by a free-radical attack [13].

Due to the ubiquitous distribution dolichol may be expected to serve essential functions in mammalian cells. At present, however, role(s) and metabolism of this lipid molecule are not fully understood [14]. It is known that dolichyl phosphate and pyrophosphate (a 1-to-20% fraction of the global dolichyl pool, the vast majority retaining the hydroxyl function in a free form or esterified with fatty acids [4]) are essential factors in the N-linked protein glycosylation in the endoplasmic

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reticulum [15]. Furthermore, studies have shown that changes in dolichol biosynthesis leading to impaired dolichol-linked oligosaccharide assembly, are involved in many congenital disorders of glycosylation [16]. No enzymatic pathway for dolichol catabolism has been reported [17]. Several experiments showed that tissue dolichol may derive from local biosynthesis and that relocation of dolichol *via* circulation cannot be prominent *in vivo* [18, 19].

On account of the ascertained susceptibility of dolichol to react with free radicals [20-22], we previously suggested that dolichol catabolism could be intertwined with the mechanism of lipid peroxidation occurring *in vivo* and possibly with antioxidant defences of eukaryotic organisms [23].

In this research, we substantiate further this hypothesis by presenting data on the effects of oxidative stress, induced by UV-B radiation, on peroxidation of lipids and levels of dolichol, coenzyme Q, α-tocopherol and PL PUFA in isolated rat hepatocytes. Our results show that even in these model conditions, the biomarker of aging dolichol has a significant role in the complex antioxidant defence strategy of cells, supporting views of a general connection between dolichol metabolism and oxidative stress.

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Materials and methods

Chemicals

All reagents were of analytical and HPLC grade. Solvents were purchased from Panreac Química S.L.U. (Barcelona, Spain). Standard molecules and chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Milli-Q (Millipore-Lab, Bedford, MA) purified water was used for all analyses.

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Animals

All of the experiments were conducted following the Official Italian Regulation n. 116/92 for the care and use of laboratory animals and were approved by the Independent Ethics Committee of the University of Pisa (Approval number: 2A/42155).

The liver samples were obtained from 3-month-old male Sprague-Dawley rats raised at the Pisa University Interdepartmental Research Centre on Biology and Pathology of Aging vivarium. The animals were kept in a controlled environment (22 °C, 12/12 h light/dark cycle) and were maintained on a standard laboratory diet (Teklad, Harlan, Italy). All rats had free access to water.

On the day of experiment, rats were sacrificed after pentobarbital anaesthesia (50 mg/kg body weight i.p.) and the liver was removed.

Preparation of isolated liver cells

Isolated hepatocytes were prepared by the method of collagenase perfusion [24]. Cell viability was tested by trypan blue exclusion and was always higher than 90 %. Cells were washed with washing buffer and suspended in Krebs-Ringer bicarbonate buffer fortified with pyruvate (15 mM) [24]. Final concentration of hepatocytes suspension was 6 mg wet cell weight ≃ 0.75 x 10^6 cells/mL. Aliquots of the cell suspension were incubated with or without mevinolin or 6-hydroxy-

2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) for approximately 10 min at room temperature and then exposed to UV-B radiation.

Ultraviolet-B (UV-B) radiation

UV-B radiation of hepatocytes was performed as described by [21]. A cell monolayer was obtained by pouring 50 mL of 6 mg wet cell weight/mL cell suspension in a Petri dish with a surface of 150 cm^2 (no cover top). The dish was placed under the radiation source for 5, 10, 20 and 40 min. At the end of radiation times, cell viability was always higher than 70% and incubation with mevinolin or Trolox had no additional effect on cell viability.

The radiation source was a flat-bed chamber for cell irradiation, designed and built by the Laboratory of Biophysics Institute of the National Research Council (IBF-CNR), Pisa. UV-B sources were two UV-B-313 fluorescent tubes, mounted about 7.0 cm above the sample holder. UV-B irradiance was about 1.7 W/m^2, slightly lower than the UV-B present in a natural environment. Light irradiance was measured by means of a United Detector Technology radiometer (UDT instruments, San Diego, CA, USA). After centrifugation of the cell suspension, the supernatant was used to assay the release of thiobarbituric acid reactive substances (TBARS) in the medium and cells were used to study the effects of radiation on dolichol, coenzyme Q, α-tocopherol and PL PUFA levels.

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Detection of ROS production using 2’,7’-dichlorodihydro-fuorescein diacetate (DCFH-DA)

ROS production was detected by using DCFH-DA. DCFH-DA is a cell-permeant non-fluorescent compound that is rapidly oxidized to a highly fluorescent form by intracellular peroxides [25, 26]. DCFH-DA cannot be added or incubated prior to UV-B radiation due to its rapid oxidation by UV-B radiation. In addition, the production of fluorescent DCFH-DA is known to be affected by incubation temperature [27, 28]. Therefore incubation conditions, including temperature and minimal ambient light, were carefully kept under control. DCFH-DA (final concentration 1 μM from stock solution of 2

mM in ethanol) was added to the sample immediately after exposition to UV-B radiation. Cells were incubated on a shaker at room temperature in the dark for 30 min. The fluorescence of the samples was measured with a spectrofluorometer (RF-5000, Shimadzu, Kyoto, Japan) at room temperature, with an excitation wavelength of 485 nm and measuring emission intensity at 520 nm to determine the relative ROS production. The results were corrected by subtracting the fluorescence of dark-kept control samples and were reported as arbitrary units. The experiments were run in three or more replicates to obtain statistically reliable data.

Thiobarbituric acid reactive substances (TBARS) assay

TBARS were assayed by taking 1mL aliquot of the medium at the end of radiation. Assay was performed as described in [29]. The quantification of TBARS production was analysed by high performance liquid chromatography-VIS detection (532 nm) using a reverse-phase column and eluted as described in [30]. The calibration curve was prepared with malondialdehyde (MDA) and the results were reported as MDA equivalents. The relative retention time for MDA was 1.8 min and total chromatographic run was 8 min.

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Lipid extraction and fatty acid analysis

Lipids were extracted with a chloroform/methanol (2:1, by vol) mixture containing 0.05 mg/mL butylated hydroxytoluene as an antioxidant following the method described in [31].
Phospholipids (PL) were separated from neutral lipids and glycolipids by Sep-Pak silica gel cartridges (Supelco, Sigma-Aldrich) by elution with chloroform/acetic acid (100:1, by vol), acetone/acetic acid (100:1, by vol) and methanol/chloroform/water (100:50:40, by vol) for neutral lipids, glycolipids and PL, respectively [32].
PL were transesterified with 5 % H_2SO_4 in methanol at 80 °C for 180 min. After esterification the fatty acid methyl esters (FAME) were extracted in hexane, dried under N_2 and redissolved in 50 μl hexane. 2 μl were used for gas-liquid chromatography (GC) analysis [33]. GC separation was

performed with a SP2340 capillary column (Supelco 60 m x 0.25 mm I.D., film thickness 0.20 μm) by a gas-liquid chromatograph (Shimadzu GC-17A) equipped with a flame ionization detector. Helium was used as the carrier gas, the flow rate was 0.9 mL/min and split ratio 1:100. The injector and detector temperatures were 250 °C and 260 °C, respectively. Initial column temperature (185 °C) was maintained for 25 min, temperature was increased at a rate of 10 °C/min to 205 °C, maintained at 205 °C for 1 min and increased at a rate of 5 °C/min to 215 °C. The final temperature was maintained for 12 min. The identities of the peaks were verified by comparison with the retention times of standard FAME. Fatty acids (FA) composition is expressed as percent of total FA.

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Extraction of dolichol, coenzyme Q and α-tocopherol

Dolichol, coenzyme Q (CoQ) and α-tocopherol were extracted simultaneously into hexane from a sodium dodecyl sulphate-treated homogenate [34]. Aliquots of the extract were taken for dolichol, CoQ assay and α-tocopherol dried under nitrogen and redissolved in isopropanol, methanol/reagent alcohol solution (ethanol/isopropanol 95/5, v/v) and methanol, respectively. Results are given as μg dolichol/g wet cell weight, μg coenzyme Q/g wet cell weight and nmol α-tocopherol/g wet cell weight. Absolute values in not-radiated cells were: dolichol 0.29 ± 0.02 pg/cell; α-tocopherol 0.26 ± 0.02 fmol/cell; CoQ9 0.84 ± 0.01 pg/cell; CoQ10 0.04 ± 0.00 pg/cell.

High-pressure liquid chromatography (HPLC) assay

Dolichol was assayed by an HPLC-UV detection (210 nm) using a reverse-phase column and eluted as described in [35]. Coenzyme Q was assayed by an HPLC-UV detection (275 nm) using a reverse-phase column and eluted as described in [34]. α-tocopherol was assayed by an HPLC-fluorometric detection (excitation at 295 nm, emission at 350 nm) using a reverse-phase column and eluted as described in [36].

Statistical analysis

The analysis of variance (ANOVA) test was used to evaluate differences among multiple conditions. If positive, the Tukey test was used to test for their statistical significance. Values of $p < 0.05$ were considered to be statistically significant.

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Results

In our setup, we tested the effect of UV- B induced oxidative stress on isolated rat hepatocytes by a toolbox of analytical techniques aiming at the determination of generated ROS and their effect on lipid peroxidation with particular regard to dolichol. No significant difference was observed on cell viability in our experimental conditions between not-radiated and radiated cells (40 min: no UV-B 78% ± 1; UV-B 74% ± 1). The decrease in cell viability after isolation had no significant effect on TBARS release in the incubation medium and on levels of dolichol and lipid soluble antioxidants in cells.

At first, to test whether UV-B radiation induced the generation of ROS in our experimental conditions, isolated liver cells were exposed to the radiation source for different times and DCFH-DA fluorescence was measured. Fig. 1 shows that UV-B radiation significantly promoted fluorescence in 5 min and levels increased linearly with UV-B radiation time for at least 10 min and saturated thereafter.

Next, we measured the production and release of TBARS in the incubation medium to assess the occurrence/extent of lipid peroxidation. As depicted in Fig. 2, TBARS levels increased almost linearly and significantly with UV-B radiation time.

More quantitative details on lipid peroxidation were obtained by the analysis of FA composition of liver PL after UV-B radiation (Table 1). We found out that saturated, monounsaturated (including the n-9 and n-7 series), diunsaturated and the ratio between mono and diunsaturated fatty acids were not affected significantly by radiation source. Conversely, a significant 10-15 % decrease in 20:4n-6 and 22:6n-3 FA was observed after 40 min of UV-B exposition. These results suggested that fat soluble antioxidants probably shielded PL PUFA from free radical attack.

Indeed, as depicted in Fig. 3, α-tocopherol content was rapidly and significantly affected by UV-B radiation: 40% of the α-tocopherol content in liver cells was depleted over the first 10 minutes, depletion was larger than 50 % in 20 min and 65 % by 40 min. Decrease of tocopherol pool was not prevented by 10 μM added Trolox (concentration similar to the concentration of α-tocopherol in cells, data not shown) and was almost fully prevented by a much higher (10 mM) concentration

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(Fig. 4a). Trolox is a water-soluble analogue of α-tocopherol with a chromanol structure which provides the antioxidant activity. Trolox was shown to give protection against oxidative reactions in aqueous solutions [37], to inhibit DNA damage induced by singlet oxygen [38] and to protect red blood cells during photodynamic treatment [39].

Also CoQ9 and CoQ10 were rapidly and significantly affected by UV-B radiation: CoQ9 and CoQ10 changes showed the same trend of α-tocopherol depletion, although the slowing of depletion kinetics observed for α-tocopherol at longer times was not detected (Fig. 3).

Finally, we assessed the temporal pattern of dolichol levels after UV-B exposition. Fig. 3 shows that UV-B radiation caused a significant decrease in the levels of liver cell dolichol (-20 %) in less than 10 min. With longer radiation times, the rate of the net loss of dolichol became smaller (-30 % in 20 min and reached a plateau thereafter). When compared with α-tocopherol, the temporal pattern of the UV-B induced changes in dolichol was similar, but lower depletion degrees were reached. As expected, in view of the different locations of dolichol and Trolox molecules inside the lipid leaflets, dolichol loss was not prevented by 10 mM added Trolox (Fig. 4b).

Mevinolin is an agent which competitively inhibits the enzyme HMGCoAR at concentrations of less than 10^{-8} M [10]. Mevinolin had no significant effect on dolichol and cholesterol levels in non-radiated cells (0 min: 32 ± 1 μg dolichol/g wet cell weight, 3.0 ± 0.1 mg cholesterol/g wet cell weight; 40 min: 30 ± 1 μg dolichol/g wet cell weight, 2.9 ± 0.1 mg cholesterol/g wet cell weight).

Addition of mevinolin to the incubation medium magnified the decrease in dolichol levels after 20 min and 40 min of UV-B radiation (Fig. 5) and increased TBARS production (Fig. 6).

Mevinolin had no significant effect on α-tocopherol depletion in radiated cells (data not shown).

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DISCUSSION

The so-called civilization diseases have numerous causes [40], the oxidative stress (OS) more or less contributes to their development. UV radiation is an easy, very well controllable and highly reproducible procedure to induce the generation of ROS and the development of oxidative stress, when formation exceeds the antioxidant defence ability of the target cell [41]. This photochemical generation of ROS is produced at random in cell hydrophilic and hydrophobic environments [42] and was shown to cause an early depletion of α-tocopherol and coenzyme Q in mice skin [43] and in a model of human skin, preceding the accumulation of altered protein [44].

In this research we explored the effects of the UV-B induced oxidative stress on dolichol levels, compared with the effects on coenzyme Q, α-tocopherol and PL PUFA. Our previous studies have already revealed a link between this biomarker of aging and free radical metabolism. ^{1}H-NMR investigation showed that dolichol protect polyunsaturated fatty acids from photoperoxydation with a sigmoidal relationship between the protection and dolichol concentration, thus indicating possible cooperativity among the lipid species during oxidative free-radical attack [20]. Furthermore, data have also shown that dolichol could be an innate, unusually efficient and promising screen for skin protection because, after UV radiation, increases its UV absorption capability extending its UV filtering range [22].

Results show that the effects of UV-B radiation were almost immediate and highly significant and caused an increase in ROS production (in much less than 10 min), in TBARS release (in a linear fashion for more than 10 min), but no significant decrease in polyunsaturated fatty acids until after 40 min, suggesting that PL PUFA are very well protected by fat soluble antioxidants. Indeed, as expected, the depletion in fat soluble antioxidants (CoQ and α-tocopherol) followed oxidative stress and preceded changes in PUFA. UV-B treatment resulted in the loss of dolichol, CoQ and α-tocopherol, and the addition of mevinolin, an inhibitor of HMGCoAR, further decreased the content of dolichol. Interestingly, the effect of mevinolin was observed only after UV-B exposition (no significant effect on dolichol content in non-radiated cells in our experimental setup, data not shown). Thus, the synergistic effect of UV-B and mevinolin on the content of dolichol cannot be

explained by a mevinolin-mediated inhibition of HMGCoAR activity but it might rather result from the oxidative status of the cells. Their redox balance was damaged by UV-B and perhaps further affected by mevinolin. It has already been shown that statin (simvastatin) increased ROS production and MDA level in lung cancer cells [45]; therefore statin might act in concert with UV-B to produce ROS which further degrade dolichol. Meanwhile oxidative stress results in an activation of HMGCoAR [46]. Taken together the mechanisms underlying observed decrease of dolichol level seem far more complex and require more studies. Notably, the addition of mevinolin (and the parallel increase in dolichol depletion) was associated with an enhanced TBARS release in the medium, therefore it is reasonable to suggest that high levels of dolichol are required for optimum protection from lipid peroxidation of PUFA-containing phospholipids.

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Molecules that prevent other molecules to be oxidized are said to be antioxidants. In this perspective, it appears that dolichol is a molecule highly prone to oxidation that behaves like a fat-soluble antioxidant. Fedorow et al. [47] have shown that dolichol is the major lipid component of neuromelanin derived from the human nervous system and suggest that dolichol may play a protective role mitigating the lipid peroxidation observed in Parkinson's disease.

Furthermore, Ward et al. [48] have observed oxidation products of dolichols in neurologically normal human autopsy brain tissue. Recently, dolichol-like compounds with a terminal-epoxide structure, which resemble brain oxidized dolichols, have been detected in the skipjack tuna liver [49]. Because an unusually long hydrophobic chain portion of dolichol or dolichol derivatives is expected to affect the bilayer membrane fluidity and permeability by skewing the fatty acid structures [50], the variations in the dolichol and oxidized dolichol derivative content might influence activity of cellular membranes [51].

However, chemical structure says that dolichol cannot be reversibly cycled between an oxidized and reduced state inside cells, as most antioxidants including coenzyme Q and vitamin E do. Previous results in vitro pointed to the same conclusion [20]. Thus dolichol could play the role of a sacrificial target of oxidation. On the other hand, our results show that the degraded dolichol may be replaced rapidly thanks to the free-radical mediated activation of HMGCoAR [13]. In view of the estimated half life of dolichol in the liver of young Sprague-Dawley rats (about 100 h) [52], it

is not conceivable that this significant depletion could be justified by a decrease in the rate of synthesis: the intensification of the loss of dolichol by mevinolin may rather be suggestive of a compensatory increase in the rate of synthesis by UV-B radiation, probably due to ROS induced HMGCoAR full activation.

Probably, dolichol depletion might result from a free-radical action on the molecule with formation of oxidized products [48] together with many possible still uncharacterized dolichol derivatives. Previously data have shown a rapid decrease of dolichol in erythroclastic organs after the administration of phenylhydrazine and it has been suggested that this depletion might be attributed to a free-radical mediate decomposition of dolichol by phagocytic cells during erythrophagocytosis [53]. Furthermore, a study show that short- and long-chain polyisoprenoid alcohols are prone to thermo-oxidation and it has been speculated that oxidized isoprenoids might be formed in the cells because of chemical processes driven by oxidative agents (e.g., reactive oxygen species) [54]. This hypothesis has recently been substantiated by experimental data indicating the susceptibility of polyisoprenoid alcohols to various oxidizing agents for the first time [55]. Yet, the full mechanisms of dolichol oxidation have to be clarified further.

The lack of any protective effect of the added Trolox may not be surprising in view of its hydrophilicity that prevents a massive access to the inner bilayer where dolichol resides [56]. Our results show that both dolichol and coenzyme Q are lost almost simultaneous with a similar (though not identical) magnitude. On account of the similarities between the polyisoprenoid chains, we may speculate that dolichol and coenzyme Q degradation may occur by the same mechanism, as previously suggested [57]. It is known in the literature that during lipid peroxidation CoQ may be broken down, the isoprenoid being drastically shortened and the intact quinone ring excreted into urine and faeces [58].

The peculiar localization of dolichol inside the bilayer, which is strictly connected to its extremely hydrophobic nature, deserves careful attention. What is the role of a free radical scavenger placed right inside the bilayer? Two different not mutually exclusive hypotheses are reasonable: a) dolichol acts as a non selective scavenger of free radicals generated deeply inside the leaflet such as with xenobiotics [21, 59]; b) dolichol acts like a free radical collector from PUFA

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and subsequently as a connecting cable feeding CoQ for final disposal [23]. The second hypothesis highlights the relevance of the peculiar three-dimensional structure of dolichol within the membrane [50].

In conclusion, both hypotheses may deserve further investigation. However, our findings show that dolichol may have a role in free-radical metabolism in cell membranes: they give support to the hypothesis that the accumulation of this biomarker of aging in older age might help to protect older membranes from an increase in free radical attack and signal an age-related alteration in the balance between ROS formation and scavenging that might make the "cellular clocks" go faster. With regard to mechanisms of dolichol accumulation in older cells, a lowered degradation rate of HMGCoAR in old cells [11] and its age-related constitutive activation probably mediated by an increased free-radical attack [13] might cause an increase in the rate of dolichol synthesis. On the other hand, age-related changes in the mono/biunsatutared fatty acid ratio in membrane phospholipids [60] might affect the functioning of the postulated cellular antioxidant machinery [23] and result in a lower free radical flux to dolichol with subsequent lower risk of free radical mediated decomposition of dolichol molecules. Probably, also the age-related decline of autophagic degradation of cellular membranes and organelles might contribute to dolichol accumulation in old cells [61].

Furthermore, our data might suggest a key to understanding some adverse effects of statin therapy, especially on skeletal muscle: from myositis to rhabdomyolysis have been reported, but the mechanism behind these myopathic changes is not fully understood [62].

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Conflict of interest : The authors declare that they have no conflict of interest.

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FIGURE CAPTIONS

Fig. 1 Effects of UV-B radiation on ROS production in isolated rat hepatocytes exposed to the radiation source for different times. Results represent the means ± SEM of five cases. ANOVA statistical analysis showed that effects of UV-B radiation on the generation of ROS were highly significant ($p < 0.01$). Post-ANOVA Tukey test ($p < 0.05$): * versus 0 min; $^{\#}$ versus 5 min; § versus 10 min

Fig. 2 Effects of UV-B radiation on lipid peroxidation in isolated rat hepatocytes. Results represent the means ± SEM of five cases. ANOVA statistical analysis showed that effects of UV-B radiation on the TBARS production and release in the incubation medium were highly significant ($p < 0.01$). Post-ANOVA Tukey test ($p < 0.05$): * versus 0 min; $^{\#}$ versus 5 min; § versus 10 min; $^{\circ}$ versus 20 min

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Fig. 3 Effects of UV-B radiation on dolichol, α-tocopherol, CoQ9 and CoQ10 levels in isolated rat hepatocytes. Results are given as percent changes with respect to not-radiated cells. Results represent the means ± SEM of five cases. Values in not-radiated cells were: dolichol 36 ± 2 µg/g wet cell weight; α-tocopherol 32 ± 2 nmol/g wet cell weight; CoQ9 105 ± 1 µg /g wet cell weight; CoQ10 4.9 ± 0.4 µg /g wet cell weight. ANOVA statistical analysis showed that effects of UV-B radiation on dolichol, α-tocopherol, CoQ9 and CoQ10 levels were highly significant ($p < 0.01$). Post-ANOVA Tukey test ($p < 0.05$):

dolichol: 0 min versus 10, 20, 40 min

α-tocopherol: 0 min versus 5, 10, 20, 40 min; 5 min versus 20, 40 min

CoQ9: 0 min versus 20, 40 min; 5 and 10 min versus 20, 40 min; 20 min versus 40 min

CoQ10: 0 min versus 20, 40 min; 5 and 10 min versus 40 min

Fig. 4 Effects of UV-B radiation on α-tocopherol (a) and dolichol (b) levels in isolated rat hepatocytes incubated with or without Trolox (10 mM). Results are given as percent changes with respect to not-radiated cells. Results represent the means ± SEM of five cases. Two-way ANOVA statistical analysis (UV-B radiation x Trolox):

α-tocopherol: UV-B radiation main effect: $p < 0.01$; Trolox main effect: $p < 0.01$; UV-B radiation by Trolox interaction: N.S. Post-ANOVA Tukey test ($p < 0.05$): UV-B versus UV-B + Trolox; [*] versus 0 min

dolichol: UV-B radiation main effect: $p < 0.01$; Trolox main effect: N.S.; UV-B radiation by Trolox interaction: N.S. Post-ANOVA Tukey test ($p < 0.05$): [*] versus 0 min; [#] versus 5 min

Fig. 5 Effects of UV-B radiation on dolichol levels in isolated rat hepatocytes incubated with or without mevinolin (3 μM). Results are given as percent changes with respect to not-radiated cells. Results represent the means ± SEM of five cases. Two-way ANOVA statistical analysis (UV-B radiation x mevinolin): UV-B radiation main effect: $p < 0.01$; mevinolin main effect: $p < 0.05$; UV-B radiation by mevinolin interaction: $p < 0.01$. Post-ANOVA Tukey test ($p < 0.05$): UV-B versus UV-B + mevinolin; [*] versus 0 min; [#] versus 5 and 10 min; [§] versus 20 min

Fig. 6 Effects of UV-B radiation on lipid peroxidation in isolated rat hepatocytes incubated with or without mevinolin (3 μM). Results are given as percent changes with respect to not-radiated cells. Results represent the means ± SEM of five cases. Two-way ANOVA statistical analysis (UV-B radiation x mevinolin): UV-B radiation main effect: $p < 0.01$; mevinolin main effect: $p < 0.01$; UV-B radiation by mevinolin interaction: $p < 0.01$. Post-ANOVA Tukey test ($p < 0.05$): UV-B versus UV-B + mevinolin; [*] min versus 0 min; [#] versus 5 and 10 min; [§] versus 20 min

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Fig. 1

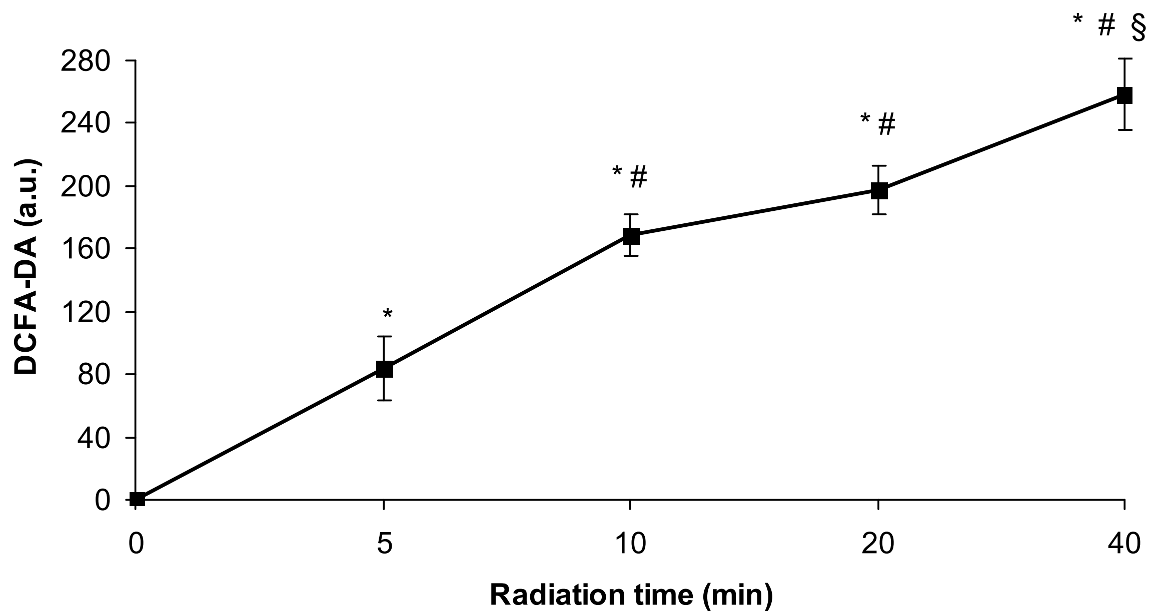

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Fig. 2

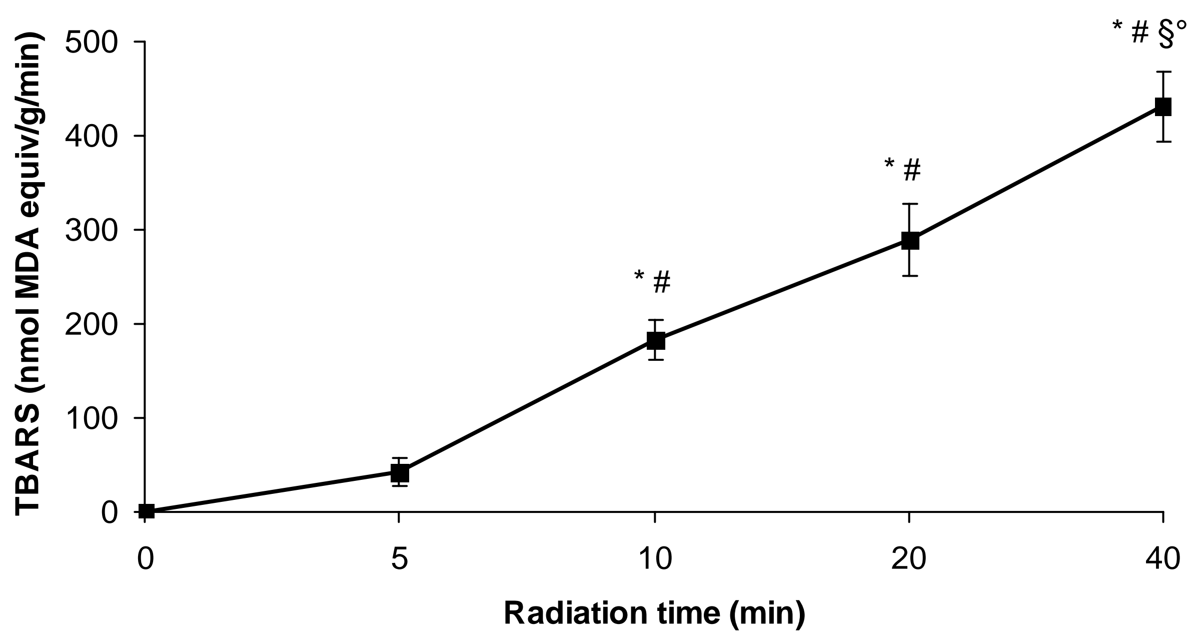

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Fig. 3

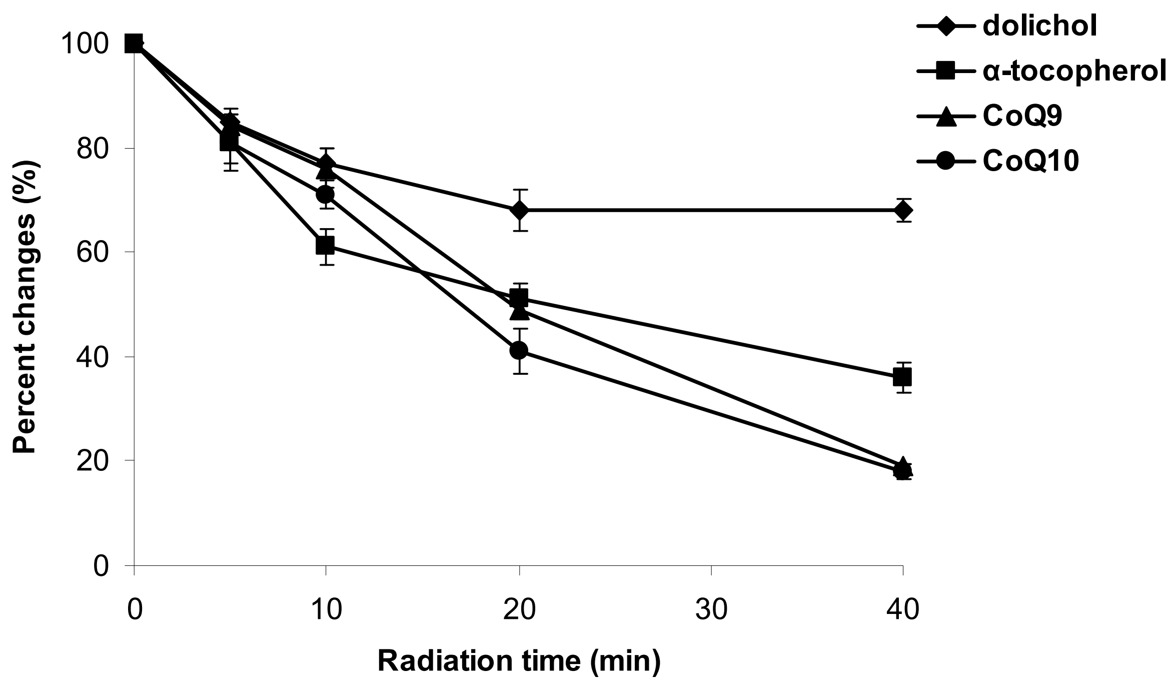

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Fig. 4

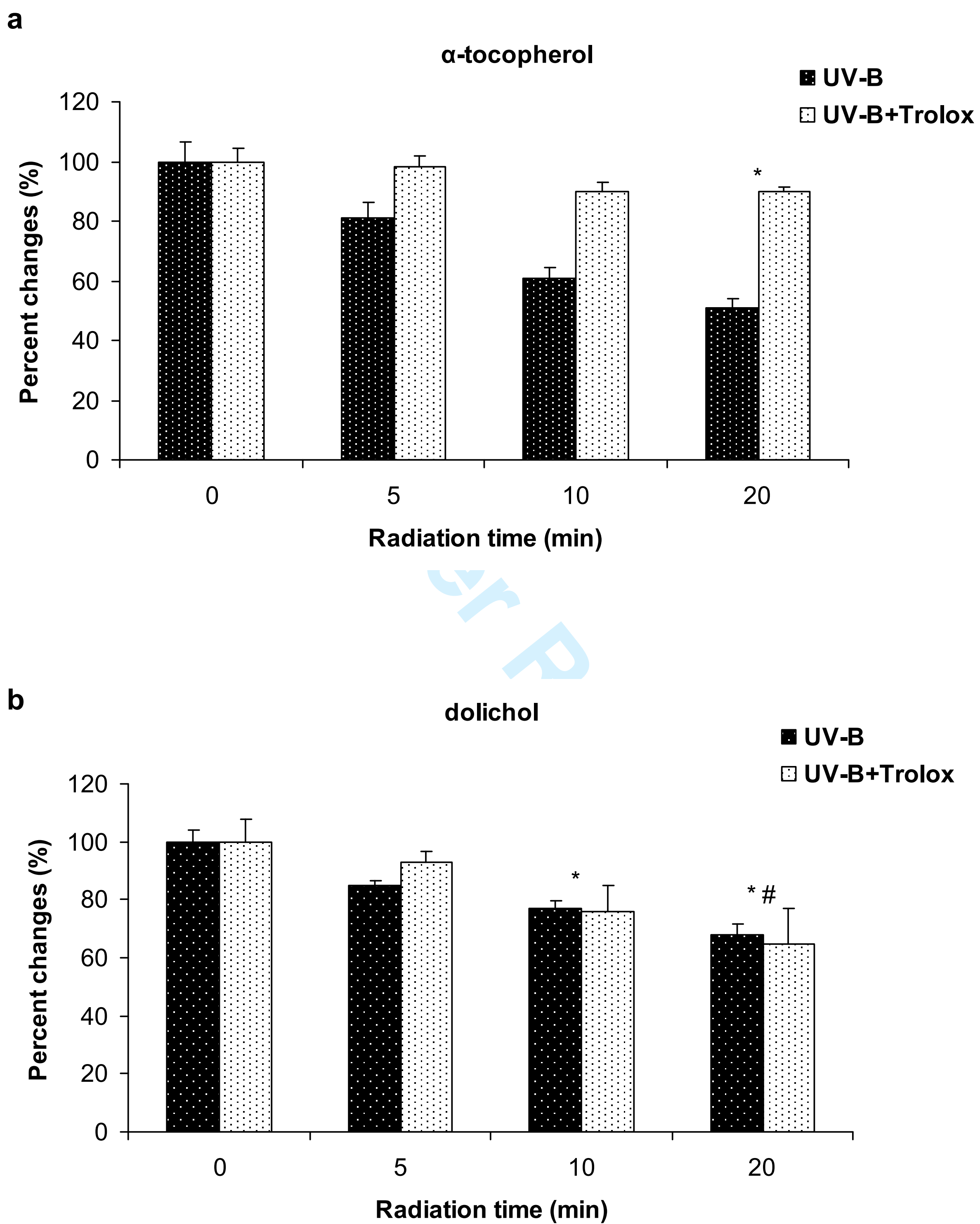

Fig. 5

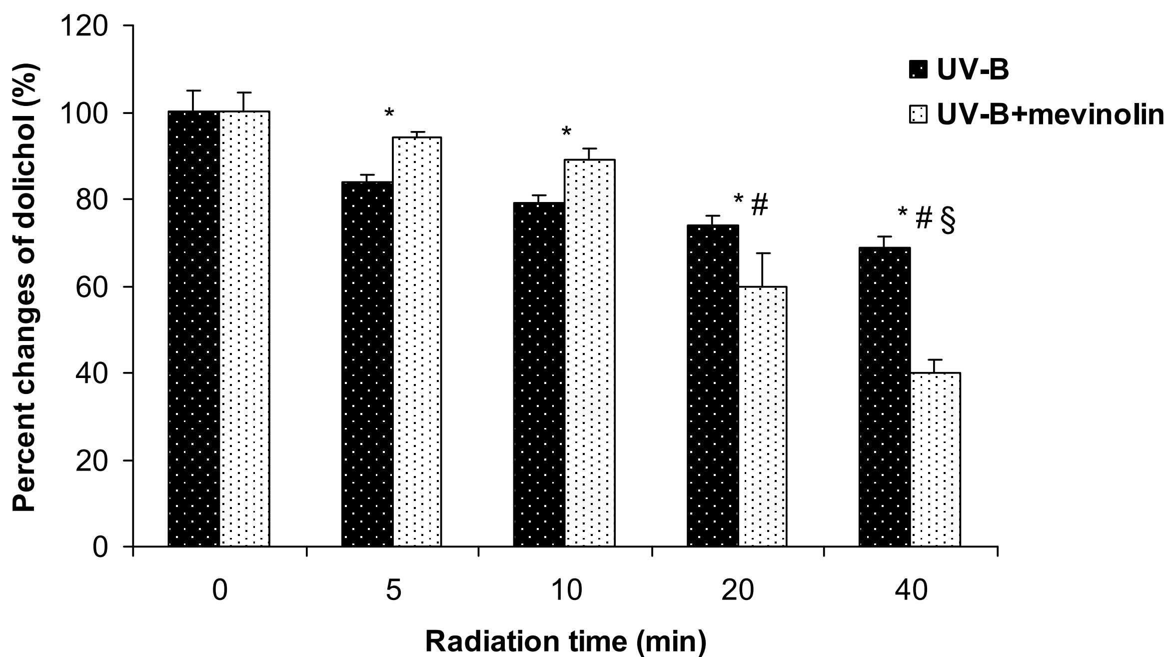

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Fig. 6

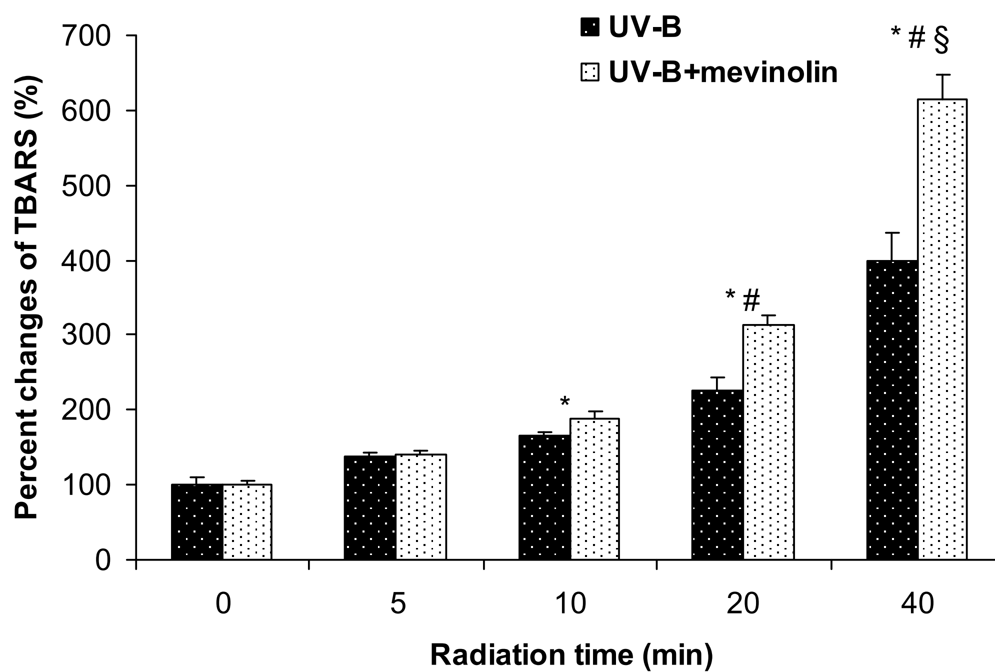

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Table 1: Effects of UV-B radiation time on the abundance of saturated and unsaturated fatty acids in liver cell phospholipids

Fatty Acids	UV-B radiation Time (min)				
	0	5	10	20	40
14:0	0.11 ± 0.01	0.17 ± 0.02	0.15 ± 0.02	0.13 ± 0.00	0.12 ± 0.00
16:0	16.90 ± 0.26	16.50 ± 0.18	16.80 ± 0.22	17.40 ± 0.21	17.30 ± 0.40
18:0	21.50 ± 0.14	21.60 ± 0.20	21.90 ± 0.12	21.30 ± 0.33	22.60 ± 0.25
20:0	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
22:0	0.22 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
24:0	0.66 ± 0.02	0.65 ± 0.04	0.67 ± 0.03	0.65 ± 0.02	0.70 ± 0.03
14:1n-5	0.18 ± 0.01	0.22 ± 0.03	0.19 ± 0.01	0.20 ± 0.03	0.18 ± 0.03
18:1n-7	2.40 ± 0.07	2.20 ± 0.01	2.30 ± 0.08	2.40 ± 0.11	2.40 ± 0.06
18:1n-9	2.20 ± 0.04	2.20 ± 0.06	2.20 ± 0.05	2.20 ± 0.06	2.30 ± 0.08
20:1n-9	0.08 ± 0.02	0.10 ± 0.04	0.10 ± 0.05	0.05 ± 0.03	0.07 ± 0.02
18:2n-6	16.30 ± 0.25	16.30 ± 0.33	16.50 ± 0.27	16.60 ± 0.32	16.60 ± 0.75
20:2n-6	0.35 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	0.34 ± 0.03	0.34 ± 0.01
18:3n-3	0.08 ± 0.02	0.07 ± 0.04	0.09 ± 0.04	0.10 ± 0.03	0.09 ± 0.01
22:5n-3	1.10 ± 0.04	1.04 ± 0.04	1.02 ± 0.03	1.07 ± 0.04	1.00 ± 0.03
22:6n-3	8.45 ± 0.25	8.15 ± 0.34	8.01 ± 0.28	7.76 ± 0.27	7.30 ± 0.22*
18:3n-6	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
20:3n-6	0.73 ± 0.06	0.61 ± 0.037	0.62 ± 0.03	0.66 ± 0.03	0.55 ± 0.02
20:4n-6	25.73 ± 0.17	25.51 ± 0.045	25.44 ± 0.25	24.56 ± 0.15	23.10 ± 0.86#

Results are given as percent composition of total FA. Results represent the means ± SEM of five cases. ANOVA statistical analysis showed that effects of UV-B radiation on the abundance of 22:6n-3 and 20:4n-6 were significant ($p < 0.05$ and $p < 0.01$, respectively). Post-ANOVA Tukey test ($p < 0.05$): * versus 0 min, 22:6n-3; # versus 0, 5 and 10 min, 20:4n-6

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