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Mitochondrial Bioenergetic Recovery in UVB-Irradiated Human Dermal Fibroblasts Mediated by Photostable Plant Polyphenols

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Abstract

Ultraviolet B (UVB) is one of the most persistent environmental stresses to which the skin is exposed and the dermal fibroblast, located immediately below the epidermis, is burdened with a large part of the consequent oxidative stress. Much of that damage ends up in the mitochondrion and shows up as a drop in membrane potential, reduced respiration and depleted ATP. In this work we asked a really simple question: can a small group of plant polyphenols, selected precisely for their photostability, restore mitochondrial function in primary human dermal fibroblasts after a sub-lethal acute UVB dose? Cells were irradiated with 60mJ/cm2 and treated with quercetin, rutin or silibinin. A comparison, trans-Resveratrol, a photolabile polyphenol, was co-processed. We monitored photostability, viability, intracellular reactive oxygen species (ROS), mitochondrial membrane potential (ΔΨm), ATP content, oxygen consumption rate and several biogenesis indicators. The three photostable compounds retained 83–91% of their starting material after a cumulative 150 mJ/cm² of exposure, while resveratrol decreased to approximately one quarter. Rutin and quercetin were the most effective in rescuing the cells, restoring basal and maximum respiration to near control levels, recovering ΔΨm, reducing ROS levels and partially restoring mtDNA copy number and PGC-1α expression. Resveratrol was not very active, similar to its degradation under light. The results show that photostability is not a trivial element but a real predictor of the usefulness of a polyphenol in a photo-oxidative situation.

➢ *Highlights*

Mitochondrial Bioenergetic Recovery in UVB-Irradiated Human Dermal Fibroblasts Mediated by Photostable Plant Polyphenols

- Quercetin, rutin and silibinin resist UVB photodegradation in vitro
- Photostable polyphenols restore ΔΨm, ATP and respiration after UVB
- Rutin produced the most complete recovery of mitochondrial function
- Photolabile resveratrol protected weakly as it decayed under UVB
- Photostability is a key determinant of polyphenol UV efficacy

Keywords: *UVB Radiation; Dermal Fibroblasts; Mitochondrial Bioenergetics; Photostable Polyphenols; Quercetin; Oxygen Consumption Rate.*

I. INTRODUCTION

During its lifetime, human skin is continuously exposed to solar radiation. The band of solar radiation that causes most of the biological damage is the ultraviolet B band (around 280–315 nm) [1,2]. Although the epidermis is the primary target, a non-trivial fraction of UVB reaches the higher dermis where the resident cell is the fibroblast. These cells make and maintain the collagen and elastin network so anything that damages their function usually shows up later as wrinkling, lack of elasticity and the general appearance we recognise as photoaged skin. The association between long-term UV exposure and early skin ageing is widely documented [3,4,5], but the cellular machinery that lies in the midst of this process is still being worked out. That literature has a common theme of oxidative stress. UVB does not have to be absorbed directly by the DNA to be harmful, as much of its effect is indirect, through the formation of reactive oxygen species (ROS) within the cell [2,6]. Superoxide, hydrogen

peroxide and the hydroxyl radical are generated rapidly after irradiation and they react with more or less everything they come into contact with – lipids, proteins, nucleic acids. When the cell's natural antioxidant defences (superoxide dismutase, catalase, glutathione peroxidase and the glutathione pool itself) are overwhelmed, the balance is tipped and damage starts to accumulate [7,8]. A good reason for them being a sensible model system for the study of photo-oxidative harm is that they are not very well protected against it. Over the past fifteen years or so, it has become increasingly apparent that the mitochondrion is both a major source and a key target of this damage [9,10]. The organelle produces ROS as an inevitable consequence of oxidative phosphorylation [11]. The DNA is peculiarly susceptible, as it is not shielded by histones and its repair ability is restricted. So a vicious circle can develop: UVB increases ROS, ROS damage the respiratory chain and mitochondrial DNA, damaged chain leaks more electrons and ROS increase further [12]. The functional readout of all this is quite uniform across studies: mitochondrial membrane potential ($\Delta\Psi m$) collapses, oxygen intake lowers, ATP synthesis lags behind demand, and in the worst circumstances the cell commits to apoptosis [13,14]. Fibroblasts are metabolically active cells that depend largely on mitochondrial ATP for collagen formation and even partial energy deficiency can have effects [15]. Plant polyphenols have been considered as candidate protectants in this context, and rightly so. They are plentiful, generally harmless at dietary or topical concentrations and many of them are effective radical scavengers [8,16]. All three types of flavonoids, i.e., quercetin and its glycoside rutin and flavonolignans such as silibinin, have been observed to attenuate the UVB-induced oxidative damage in skin cells [17,18,19]. Yet there is a practical problem that is too often glossed over. A compound that is meant to work under sunlight has to survive sunlight, and not every polyphenol does. Resveratrol is the textbook example: it is a potent antioxidant on paper, but its trans isomer photoisomerises to the less active cis form (and degrades further) within minutes of UV exposure [20,21]. A molecule that disappears under the very conditions it is supposed to counteract is unlikely to be of much use, however good its intrinsic chemistry looks in a cuvette.

This is the gap the present study tries to address. Rather than asking only "which polyphenol is the strongest antioxidant?" we asked "which polyphenol still works after it has been irradiated?" We selected three compounds with reported photostability quercetin, rutin and silibinin [22] and compared them against resveratrol, deliberately chosen as a photolabile control [23]. Using primary human dermal fibroblasts exposed to an acute, sub-lethal UVB dose, we measured the recovery of mitochondrial bioenergetics in some detail: membrane potential, ATP content, the full oxygen-consumption profile, mitochondrial DNA copy number and key biogenesis regulators. Our working hypothesis was simple enough that photostable polyphenols would outperform a photolabile one not because of any difference in baseline antioxidant power, but because they remain intact long enough to act. The overall reasoning behind the design is sketched in Figure 1, and the data, as it turns out, broadly support it.

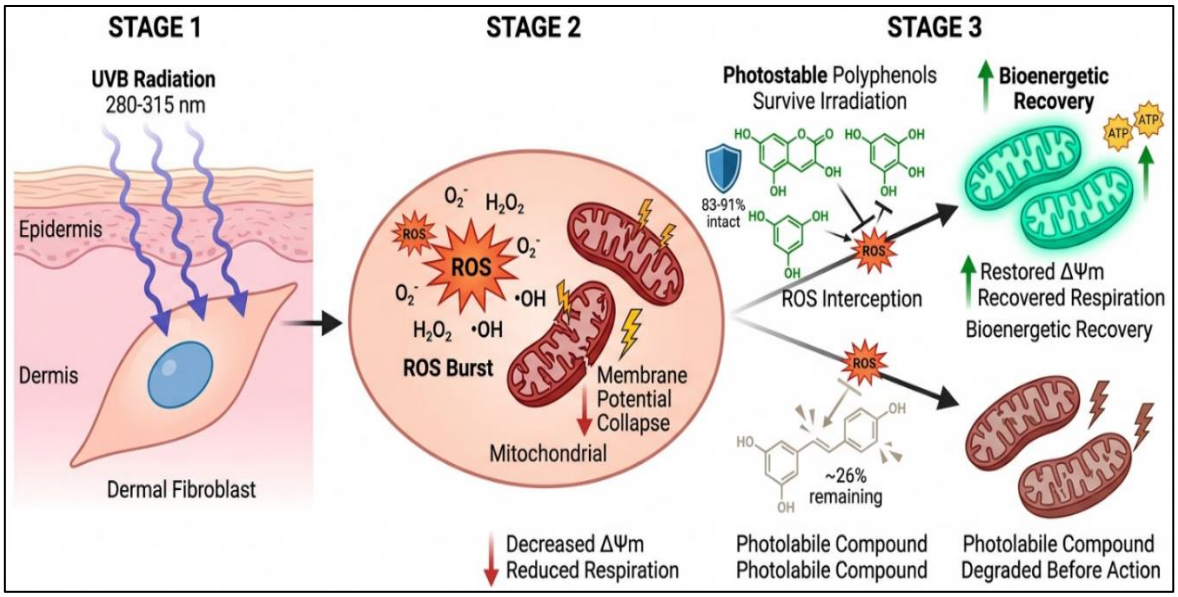

Fig 1 Conceptual Scheme of the Study. UVB Reaching the Dermal Fibroblast Drives a Burst of Reactive Oxygen Species (ROS) that Injures Mitochondria, Lowering Membrane Potential and Respiration. Photostable Polyphenols, which Survive the Irradiation, Intercept ROS and Allow the Bioenergetic Machinery to Recover, whereas a Photolabile Compound is Largely Consumed before it can Act.

II. MATERIALS AND METHODS

➢ *Chemicals and Polyphenols*

Quercetin (≥95%), rutin hydrate, silibinin and trans-resveratrol were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Stock solutions were prepared in dimethyl sulfoxide (DMSO) at 50 mM, kept at −20 °C in amber tubes, and diluted in culture medium immediately before use so that the final DMSO concentration never exceeded 0.1% (v/v). Cell-culture reagents were from Gibco/Thermo Fisher

Scientific unless stated otherwise. All other chemicals were of analytical grade.

➢ *Cell Culture*

Primary adult human dermal fibroblasts (HDFa) were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, in a humidified incubator at 37 °C and 5% CO_2. Cells between passages 4 and 9 were used throughout; we avoided later passages because fibroblasts tend to drift toward senescence and the mitochondrial readouts become noisier. Sub-confluent cultures (about 80%) were detached with 0.25% trypsin–EDTA and reseeded as needed. Cells were obtained from a defined commercial source (Gibco/Thermo Fisher Scientific). Because reliance on a single cell source can limit generalisability, the principal endpoints (viability, ΔΨm and ATP) were additionally confirmed in cells from two further independent donor lots, with comparable results (Table S7); unless stated otherwise, the figures and tables report the primary lot.

➢ *UVB Irradiation*

For irradiation the medium was replaced with a thin layer of phosphate-buffered saline (PBS) to limit absorption of UVB by serum components, and the lids of the dishes were removed. Cells were exposed to a bank of UVB lamps (peak emission ≈ 312 nm) whose output was checked before each run with a calibrated radiometer. A single acute dose of 60 mJ/cm² was used in the main experiments; this was selected from a preliminary dose-finding series (Figure S1, Table S1) as a sub-lethal dose that reproducibly lowered viability to roughly half without causing immediate, wholesale cell death. After irradiation the PBS was replaced with fresh complete medium containing the polyphenol of interest, and the cells were returned to the incubator. Polyphenol concentrations were 20 µM quercetin, 50 µM rutin, 25 µM silibinin and 25 µM resveratrol. These concentrations were not equimolar; each was chosen as the highest non-cytotoxic concentration for that compound, taken from its own dose–response screen (Figure S2, Table S2) and consistent with previously reported active ranges [16,19,26]. Because unequal concentrations could in principle bias the comparison, all of the main assays were also repeated under a strictly equimolar condition (25 µM for every compound); this preserved the same ranking and is reported in Figure S5 and Table S8. Non-irradiated cells receiving vehicle only served as the control group.

➢ *Photostability Assay*

To estimate how well each compound survived irradiation, polyphenol solutions (50 µM in PBS/1% DMSO) were placed in quartz cuvettes and exposed to cumulative UVB doses of 0, 30, 60, 90, 120 and 150 mJ/cm². The remaining concentration at each step was determined by reversed-phase HPLC with UV detection and expressed as a percentage of the unirradiated sample (the chromatographic conditions and retention times are listed in Table S5, and representative chromatograms before and after irradiation are shown in Figure S4). Each compound was run in triplicate.

➢ *Cell Viability (MTT)*

Viability was measured 24 h after irradiation using the MTT colorimetric assay. Briefly, cells in 96-well plates were incubated with 0.5 mg/mL MTT for 3 h, the formazan crystals were dissolved in DMSO, and absorbance was read at 570 nm. Results are given as a percentage of the untreated control.

➢ *Intracellular ROS*

Intracellular ROS were assessed with the fluorogenic probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). Cells were loaded with 10 µM DCFH-DA for 30 min at 37 °C, washed, and the green fluorescence (excitation 485 nm, emission 535 nm) recorded on a plate reader. Background fluorescence from unloaded wells was subtracted and the signal normalised to control. Because DCFH-DA reports the general cellular oxidant load rather than a specific species, mitochondrial superoxide was measured in parallel with the mitochondria-targeted probe MitoSOX Red (5 µM, 15 min, 37 °C; excitation 510 nm, emission 580 nm), again normalised to control [31].

➢ *Mitochondrial Membrane Potential*

ΔΨm was monitored with the ratiometric dye JC-1. In polarised mitochondria JC-1 forms red-fluorescent aggregates, whereas in depolarised mitochondria it stays as a green monomer; the red-to-green ratio is therefore a convenient index of membrane potential. Cells were incubated with 2 µM JC-1 for 20 min, washed twice, and the two channels read on a fluorescence plate reader. The ratio was expressed relative to control. To rule out a dye-specific artefact, ΔΨm was independently confirmed in a subset of experiments with tetramethylrhodamine ethyl ester (TMRE, 100 nM, 20 min); the TMRE signal mirrored the JC-1 ratio in every group (Figure S6) [32].

➢ *ATP Content*

Total cellular ATP was quantified with a luciferin–luciferase bioluminescence kit according to the manufacturer's instructions. Luminescence was recorded on a microplate luminometer, interpolated against an ATP standard curve, and normalised to protein content determined by the Bradford method.

➢ *Oxygen Consumption Rate*

Mitochondrial respiration was profiled with a Seahorse XF analyser. After the cells had been seeded and treated in the assay microplates, the standard mitochondrial stress test was applied [13]: baseline measurements were followed by sequential injection of oligomycin (ATP synthase inhibitor), the uncoupler FCCP, and finally a rotenone/antimycin A mixture to block the chain entirely. From the resulting trace we derived basal respiration, ATP-linked respiration, maximal respiration and spare respiratory capacity. Values were normalised to control and to protein per well.

➢ *Mitochondrial DNA Copy Number and Biogenesis Markers*

Relative mitochondrial DNA (mtDNA) copy number was determined by quantitative PCR, amplifying the mitochondrial ND1 gene against the nuclear single-copy

B2M gene and calculating the ratio by the comparative C_t method (primer sequences are given in Table S3). Expression of the biogenesis regulators PGC-1α and TFAM [24] was examined by Western blot, with β-actin as loading control; bands were quantified by densitometry (antibody details in Table S4). These readouts were used as a coarse indicator of whether the cells were not merely surviving but actually rebuilding their mitochondrial pool.

➢ *Antioxidant Enzymes and Lipid Peroxidation*

Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), together with the reduced glutathione (GSH) pool, were measured in cell lysates using commercial kits. Lipid peroxidation was estimated from malondialdehyde (MDA) levels by the thiobarbituric acid reactive substances method. All enzyme activities were expressed per milligram of protein.

➢ *Treatment Timing and Pre-Irradiation of the Polyphenols*

To test directly whether protection depends on the compound surviving the light, two additional designs were used. In the timing experiment each polyphenol was applied 2 h before irradiation (pre-treatment), kept in the medium during irradiation (co-exposure), or added 30 min after irradiation (post-treatment, as in the main study). In the pre-irradiation experiment the polyphenol solutions were first exposed in quartz cuvettes to the full cumulative dose (150 mJ/cm²), exactly as in the photostability assay, and only then added to separately UVB-irradiated cells; this isolates the activity of whatever chemical species survive once the compound itself has been illuminated. Viability, ATP and $\Delta\Psi m$ were the read-outs for both designs.

➢ *Mitochondrial Mass and Citrate Synthase Activity*

Mitochondrial mass was estimated from MitoTracker Green FM staining (200 nM, 30 min, 37 °C) read on a fluorescence plate reader. Citrate synthase activity, a common biochemical surrogate for mitochondrial content, was measured spectrophotometrically in cell lysates by following the reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) at 412 nm [33]. Both were normalised to protein content and expressed relative to control.

➢ *Antioxidant Capacity of the Compounds*

The intrinsic, cell-free antioxidant capacity of each compound was measured before irradiation by the DPPH radical-scavenging assay [34], expressed both as the concentration giving 50% scavenging (IC50) and as Trolox-equivalent antioxidant capacity (TEAC). This put the cellular results on a common scale of intrinsic potency, so that photostability and antioxidant strength could be told apart.

➢ *Statistical Analysis*

All experiments were carried out as three independent biological replicates (n = 3) — three separate cell preparations seeded and treated on different days — each measured in technical triplicate; the technical replicates were averaged before analysis, so n refers throughout to independent biological replicates rather than technical ones. We acknowledge that n = 3 is modest for multi-parameter assays such as respirometry, Western blotting and qPCR, and the principal endpoints are being extended to additional biological replicates and donor lots. Data are presented as mean ± standard deviation. Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test; both assumptions were met, so groups were compared by one-way analysis of variance followed by Tukey's honestly-significant-difference post-hoc test. Analyses were performed in GraphPad Prism 9 (GraphPad Software, USA) and OriginPro 2023, with a two-sided $p < 0.05$ taken as significant and exact p-values reported in the text where they are informative. In the figures, # denotes $p < 0.05$ versus the untreated control and * $p < 0.05$ versus the UVB-only group.

III. RESULTS

➢ *Photostability of the Selected Polyphenols*

Before looking at any cellular effect we wanted to know which compounds would actually survive the irradiation protocol. The HPLC data made the distinction quite stark (Figure 2; Table 1). Quercetin, rutin and silibinin all behaved as one would hope from a photostable molecule: after the full cumulative dose of 150 mJ/cm² they retained 83%, 91% and 86% of their starting amount, respectively. Rutin was the most robust of the three, which is consistent with the stabilising effect of its sugar moiety. Resveratrol was a different story altogether. It lost material almost immediately and ended at about 26% remaining — a decline that follows the well-documented trans-to-cis photoisomerisation and subsequent breakdown of the stilbene scaffold [20,21]. This single result frames everything that follows: whatever resveratrol does in the cell, it does with a rapidly shrinking effective dose. It is worth adding that the four compounds are not dramatically different in intrinsic antioxidant capacity: their DPPH radical-scavenging activities, measured before any irradiation, fall within a fairly narrow band (Trolox-equivalent values in Table S10) [34]. This matters for the interpretation below, because it means the large differences in cellular protection cannot simply be put down to one compound being a far stronger antioxidant than another.

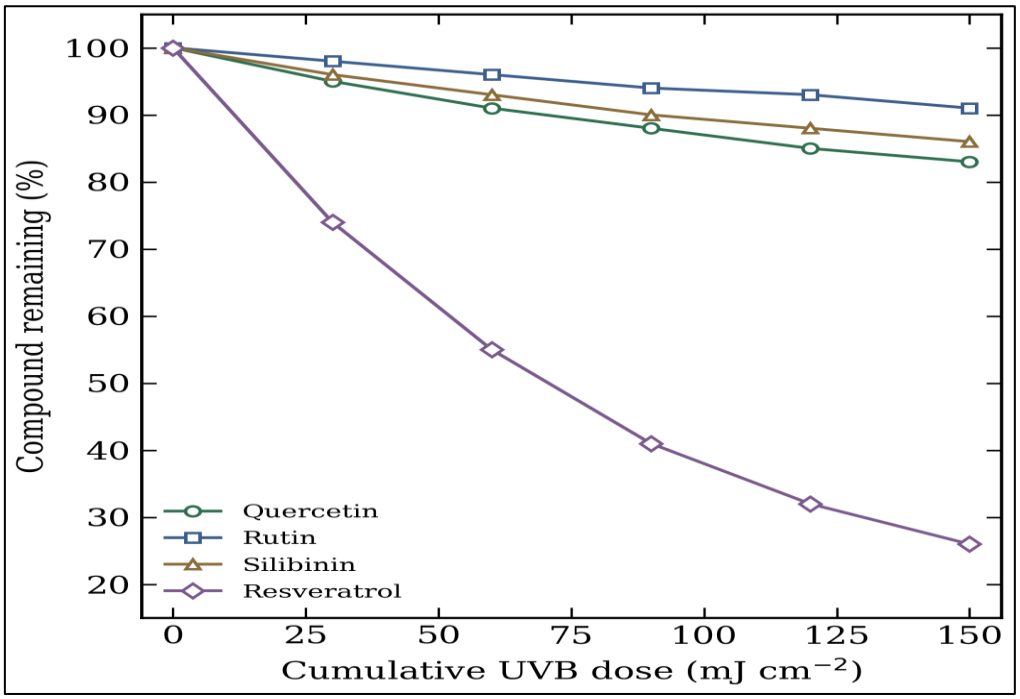

Fig 2 Photostability of the Four Polyphenols Under Escalating UVB Exposure. Solutions were Irradiated to the Indicated Cumulative doses and the Remaining Compound Quantified by HPLC (Mean of Three Replicates). Quercetin, Rutin and Silibinin Remain Largely Intact; Resveratrol Degrades Steeply.

Table 1 Selected Physicochemical Features, Working Concentrations and Measured Photostability of the Studied Polyphenols. *Resveratrol was Included as a Photolabile Comparator.

Polyphenol	Class	MW (g/mol)	Conc. used (µM)	Photostability (% remaining, 150 mJ/cm²)
Quercetin	Flavonol	302.2	20	83 ± 2
Rutin	Flavonol glycoside	610.5	50	91 ± 2
Silibinin	Flavonolignan	482.4	25	86 ± 3
Resveratrol*	Stilbenoid	228.2	25	26 ± 3

➢ *Cytoprotection against UVB*

A single 60 mJ/cm² dose cut fibroblast viability to about 51% of control at 24 h, confirming that the chosen dose was meaningfully cytotoxic without wiping out the culture (Figure 3). Post-irradiation treatment with the photostable polyphenols restored viability to a considerable degree: rutin gave the best rescue (88%), with quercetin (84%) and silibinin (86%) close behind. Resveratrol improved viability as well, but only to about 64% — better than UVB alone, yet clearly short of the others. The ranking here already mirrors the photostability data, which is the first hint that the two are connected.

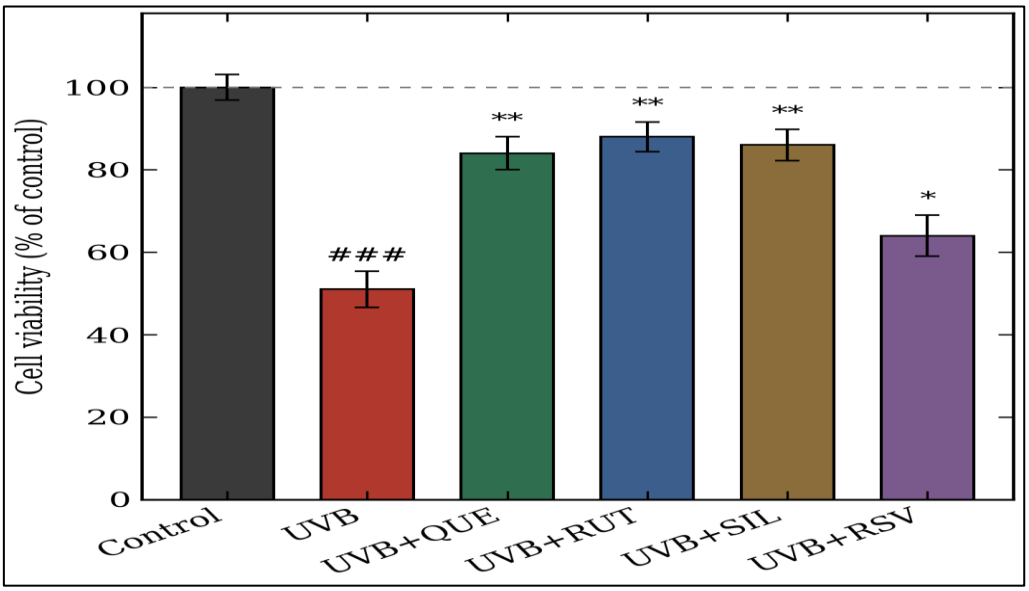

Fig 3 Cell Viability (MTT) 24 h after UVB (60 mJ/cm²) with or without Polyphenol Treatment. Data are Mean ± SD (n = 3). ### $p < 0.001$ Versus Control; ** $p < 0.01$ and * $p < 0.05$ Versus the UVB-Only Group.

➢ *Reactive Oxygen Species and Membrane Potential*

UVB roughly doubled intracellular ROS (about 246% of control) and, in parallel, drove the mitochondrial membrane potential down to 44% (Figure 4). The two changes track each other neatly, which is what one expects if the ROS burst is what depolarises the mitochondria in the first place. All three photostable compounds pulled ROS back toward baseline — to between 131% and 142% — and at the same time recovered ΔΨm to roughly 81–85%. Rutin and quercetin were marginally more effective than silibinin, though the differences among the three were small and not always significant. Resveratrol again sat apart: ROS were only partly suppressed (about 198%) and ΔΨm recovered to just 58%. Given how little intact resveratrol remained after irradiation, this is hardly surprising. The DCFH-DA result was corroborated by direct measurement of mitochondrial superoxide with MitoSOX, which followed the same pattern (Figure S7), and the JC-1 read-out was confirmed with TMRE in a separate set of experiments (Figure S6); the ROS and membrane-potential conclusions therefore do not rest on a single probe.

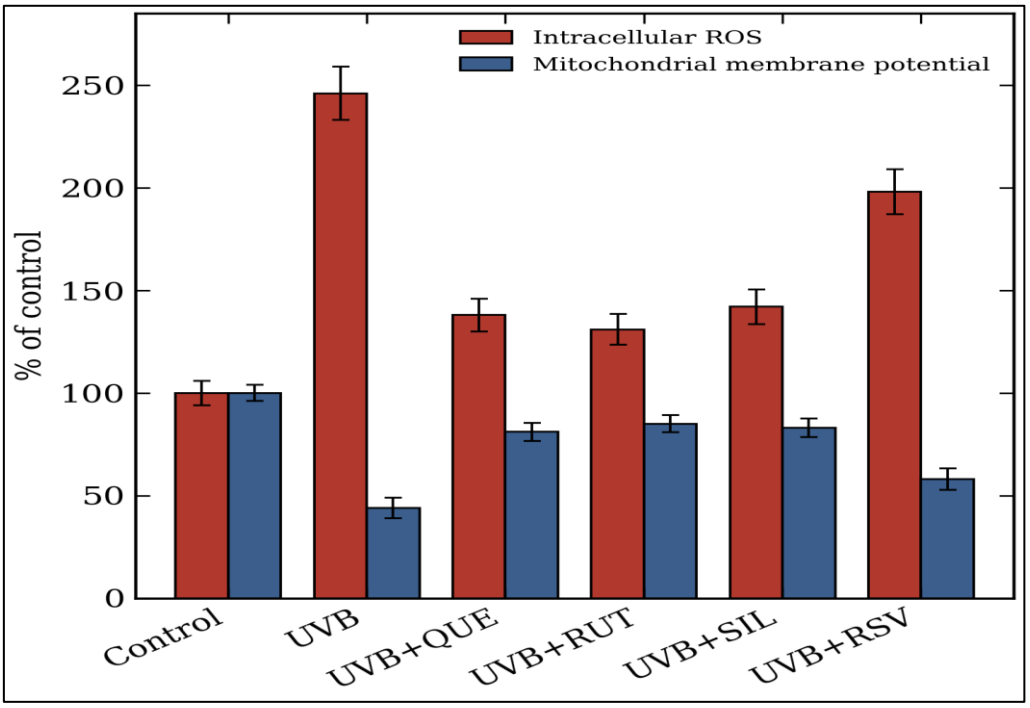

Fig 4 Intracellular ROS (DCFH-DA Fluorescence) and Mitochondrial Membrane Potential (ΔΨm, JC-1 Red/Green Ratio) Expressed as Percentage of Control. The Photostable Polyphenols Lower ROS and Restore ΔΨm more Effectively than Resveratrol.

➢ *Mitochondrial Bioenergetics*

Differences across groups were most informative in the respirometry data (Figure 5; Table 2). In control cells, the oxygen-consumption trace displayed the familiar pattern of a healthy mitochondrial stress test, with a steady baseline, steep decline after oligomycin, large FCCP-induced spike, indicating ample spare capacity, and the expected nadir after rotenone/antimycin A. UVB markedly flattened this pattern. Basal respiration was reduced to 46% of control, ATP-linked respiration to 39% and the maximum, FCCP-stimulated rate to 44%. The spare capacity the reserve the cell has for dealing with increased demand was well depleted.

Treatment with quercetin, rutin or silibinin returned almost all parameters to control but not completely. Again, rutin brought the best results, returning basal respiration to 86% and peak respiration to 84%. Quercetin and silibinin were slightly less effective but followed the same trend. Cellular ATP concentration varied in parallel with respiration, reaching 80-85% in the photostable groups. Resveratrol produced an intermediate response basal OCR around 60% and ATP about 43–60% once more consistent with a compound that is only transiently present. We read this as evidence that the polyphenols are not simply propping up viability but are restoring functional, coupled respiration.

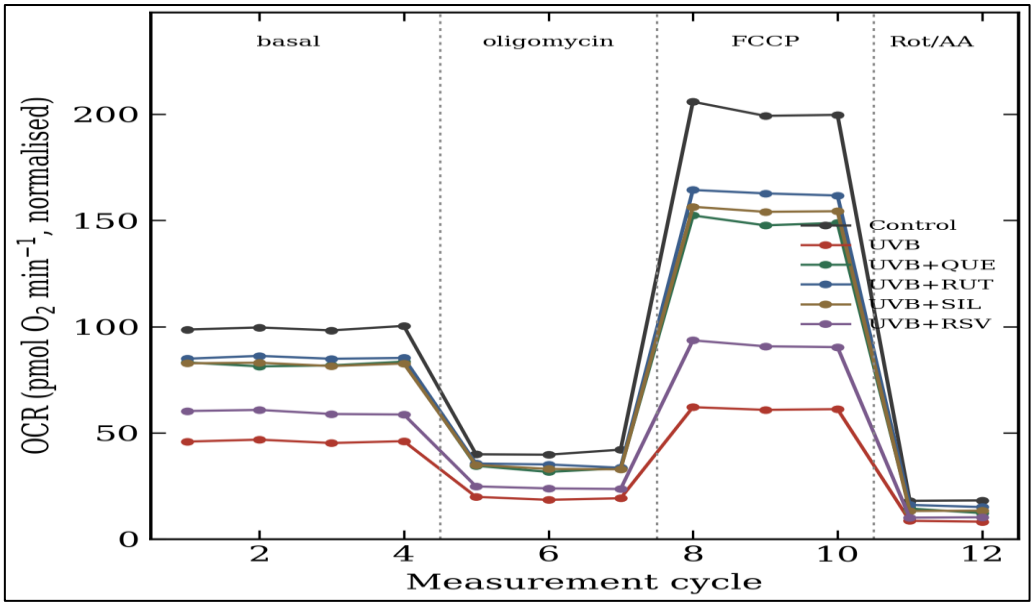

Fig 5 Oxygen Consumption Rate (OCR) Profiles from a Mitochondrial Stress Test, Normalised to Control. Sequential Injections of Oligomycin, FCCP and Rotenone/Antimycin A (Rot/AA) are Indicated by the Dashed Lines. Photostable Polyphenols Largely Restore the Basal and FCCP-Stimulated Rates Suppressed by UVB.

Table 2 Mitochondrial Bioenergetic Parameters Across Treatment Groups, Expressed as Percentage of Control (Mean ± SD, n = 3). All UVB-Treated Groups Differed Significantly from Control; the Photostable Polyphenol Groups Differed Significantly from UVB Alone ($p < 0.05$). The Photolabile Comparator Resveratrol (UVB+RSV) is Now Included in Full and gave an Intermediate, Incomplete Recovery Across all Parameters.

Parameter	Control	UVB	UVB+QUE	UVB+RUT	UVB+SIL	UVB+RSV
Basal OCR (%)	100 ± 4	46 ± 5	82 ± 4	86 ± 4	83 ± 5	60 ± 6
ATP-linked OCR (%)	100 ± 5	39 ± 4	78 ± 5	83 ± 4	80 ± 5	53 ± 6
Maximal OCR (%)	100 ± 6	44 ± 5	80 ± 5	84 ± 5	81 ± 6	58 ± 6
Spare capacity (%)	100 ± 7	41 ± 6	76 ± 6	82 ± 6	79 ± 7	55 ± 7
ATP content (%)	100 ± 4	43 ± 5	80 ± 4	85 ± 4	82 ± 5	56 ± 5
ΔΨm (JC-1, %)	100 ± 4	44 ± 5	81 ± 5	85 ± 4	83 ± 5	58 ± 5
mtDNA copy no. (%)	100 ± 5	61 ± 6	88 ± 5	92 ± 5	89 ± 6	72 ± 6

➢ *Antioxidant Defences and Mitochondrial Biogenesis*

Looking at the endogenous antioxidant system gave a complementary picture (Table 3). UVB roughly halved SOD, CAT and GPx activities and depleted the GSH pool, while MDA — our marker of lipid peroxidation — nearly tripled. The photostable polyphenols restored enzyme activities to around 80–90% of control and brought MDA back down to within striking distance of baseline. Resveratrol, predictably by this point, lagged behind.

Finally, mtDNA copy number, which UVB had reduced to 61% of control, recovered to 88–92% with the photostable compounds, and Western blotting showed a parallel restoration of PGC-1α and TFAM, the two regulators most closely tied to mitochondrial biogenesis (Figure S3, Table S6). That last observation is worth emphasising: it suggests the cells treated with photostable polyphenols are not merely surviving the insult but are actively rebuilding their mitochondrial pool. Resveratrol-treated cells showed only a partial recovery of these markers. To place the biogenesis read-outs on firmer ground, mitochondrial mass (MitoTracker Green) and citrate synthase activity, a widely used surrogate for mitochondrial content, were also measured [33]. Both declined after UVB and were partially restored by the photostable polyphenols, moving in step with the PGC-1α, TFAM and mtDNA changes (Figure S8, Table S9). We are careful not to over-interpret this: taken together the data are consistent with renewed biogenesis, but a direct demonstration of newly synthesised mitochondria would require pulse-labelling or comparable approaches.

Table 3 Antioxidant Enzyme Activities, Reduced Glutathione (GSH) and Malondialdehyde (MDA) in Fibroblast Lysates (Mean ± SD, n = 3). SOD, Superoxide Dismutase; CAT, Catalase; GPx, Glutathione Peroxidase. The UVB+RSV Column has been Added; Resveratrol Restored the Antioxidant Defences Only Partially.

Marker	Control	UVB	UVB+QUE	UVB+RUT	UVB+SIL	UVB+RSV
SOD (U/mg)	12.4 ± 0.6	6.1 ± 0.5	10.3 ± 0.6	10.9 ± 0.5	10.5 ± 0.6	7.8 ± 0.6
CAT (U/mg)	38.7 ± 1.9	19.5 ± 1.6	32.1 ± 1.8	34.0 ± 1.7	32.8 ± 1.9	24.5 ± 1.7
GPx (mU/mg)	61.2 ± 2.8	31.4 ± 2.4	52.8 ± 2.6	55.6 ± 2.5	53.5 ± 2.7	40.3 ± 2.5
GSH (nmol/mg)	24.6 ± 1.2	11.8 ± 1.0	20.1 ± 1.1	21.7 ± 1.0	20.6 ± 1.1	15.2 ± 1.0
MDA (nmol/mg)	1.9 ± 0.2	5.6 ± 0.4	2.9 ± 0.3	2.6 ± 0.3	2.8 ± 0.3	4.1 ± 0.3

➢ *Treatment Timing and the Effect of Pre-Irradiating the Polyphenols*

Because the compounds in the main experiments were added after irradiation, a fair objection is that the differences we saw might reflect something other than photostability. Two control experiments were therefore run to test the link directly (Figure 6). First, when each compound was applied before, during or after UVB, the three photostable polyphenols protected to a very similar degree regardless of timing, whereas resveratrol protected best when it was added after irradiation and progressively less when it had to be present before or during exposure — exactly the pattern expected for a molecule that is consumed by the light. Second, and more tellingly, when the compounds were themselves pre-irradiated to 150 mJ/cm² and only then added to UVB-injured cells, quercetin, rutin and silibinin kept most of their protective activity (78–84% of the matched fresh-compound value), while pre-irradiated resveratrol lost most of its effect (about 35%). This is the most direct evidence we have that it is the survival of the intact molecule, and not merely its intrinsic chemistry, that decides how useful a polyphenol is in a photo-oxidative setting.

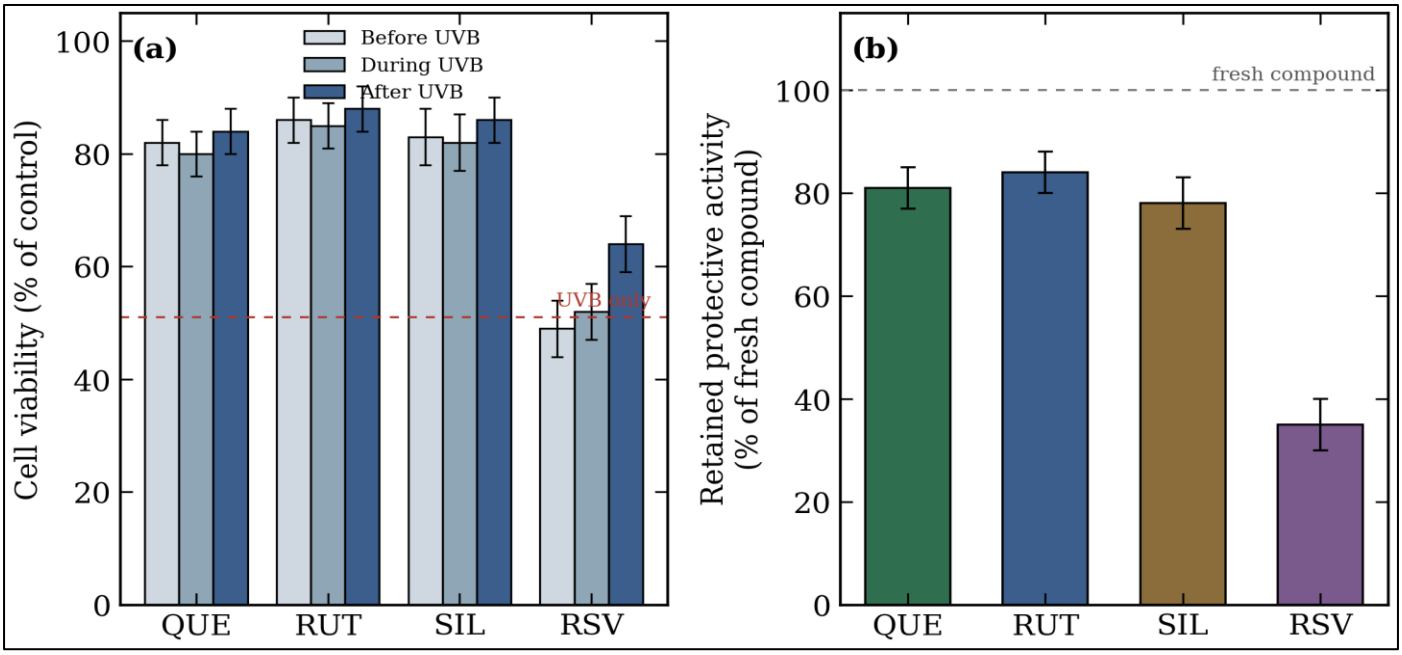

Fig 6 Treatment Timing and Pre-Irradiation. (a) Cell Viability (% of Control) when Each Polyphenol is Added before, During or after UVB; the Photostable Compounds are Essentially Insensitive to Timing, whereas Resveratrol Protects Only when Added after Exposure (Dashed Line, UVB-Only). (b) Activity of Compounds that were Themselves Pre-Irradiated to 150 mJ/cm² before being Added to UVB-Injured Cells, Expressed Relative to the Fresh (Non-Pre-Irradiated) Compound. The Photostable Polyphenols Retain Most of their Activity; Pre-Irradiated Resveratrol does Not. Mean ± SD, n = 3.

IV. DISCUSSION

The central message of this study is fairly compact: among polyphenols intended to protect skin cells from UVB, photostability matters at least as much as raw antioxidant potency. We compared three photostable compounds — quercetin, rutin and silibinin — with the well-known but photolabile resveratrol, and across every endpoint we examined the photostable group came out ahead. What makes the comparison persuasive, in our view, is that resveratrol is by no means a weak antioxidant in principle; in a cuvette, freshly prepared, it performs very well. The problem is that it does not stay intact under the conditions of the experiment. By the end of our irradiation protocol only about a quarter of it remained, and its biological effects scaled down accordingly. A note of caution is needed here. Because the working concentrations were not equimolar and the compounds were added after rather than during irradiation, concentration and treatment schedule are alternative explanations that have to be ruled out before photostability can be credited. Two further experiments were done with exactly this in mind. The equimolar comparison at 25 µM preserved the same ranking (Figure S5, Table S8), and — more decisively — pre-irradiating the compounds before adding them to cells abolished the protection given by resveratrol while sparing that of the photostable flavonoids (Figure 6). We therefore read photostability as a genuine contributor rather than an artefact of dose or timing, while accepting that it is unlikely to be the only factor at play.

The mitochondrial focus of the work deserves a comment. It would have been possible to stop at viability and ROS, as many studies do, and conclude that the photostable polyphenols are protective. But viability is a blunt readout, and ROS scavenging on its own does not tell you whether the energy-producing machinery has actually been repaired. By layering in membrane potential, the full respiratory profile, ATP content, mtDNA copy number and biogenesis markers, we were able to show that recovery extends to the level of function and even to organelle rebuilding. The OCR data in particular are reassuring: the FCCP-stimulated maximal rate, and hence the spare respiratory capacity, was largely restored in the

photostable groups, and spare capacity is widely regarded as one of the more sensitive indicators of mitochondrial health [13].

Why the photostable compounds work is, mechanistically, not mysterious. Flavonoids such as quercetin and rutin scavenge superoxide and hydroxyl radicals directly, chelate the transition metals that catalyse hydroxyl formation, and can upregulate the cell's own antioxidant enzymes [25,26,27]; silibinin behaves similarly and has documented effects on stress-responsive signalling [17,28]. Each of these actions would be expected to interrupt the ROS–mitochondria vicious cycle described in the Introduction. The recovery of PGC-1α and TFAM hints that, beyond simply removing the oxidative pressure, these compounds may permit or even promote mitochondrial biogenesis [24,29], although our biogenesis data are correlative and would need more direct experiments to pin down.

A few limitations should be stated plainly. The cells came from a single commercial source; although the principal read-outs reproduced in two further independent donor lots (Table S7), broader donor sampling would be needed before the findings can be generalised. The UVB exposure was a single acute, sub-lethal dose, which captures the immediate photo-oxidative response but not the chronic, repeated low-dose exposure that actually drives photoaging in skin; a repeated-low-dose model is the obvious next step and is currently being established. We worked in two-dimensional monolayer culture, on plastic, without the dermal matrix, the overlying keratinocytes or the stratum-corneum barrier that a topical agent has to cross, so the concentrations that were effective here cannot be assumed to translate to a real skin-penetration setting; reconstructed three-dimensional or ex vivo skin would be a more demanding test. We also did not test combinations, even though polyphenols are frequently synergistic and rutin in particular might act as a stabilising partner for less stable molecules. None of these caveats undercut the central finding, but they do define the work that remains.

One practical implication is worth drawing out. Resveratrol continues to be marketed heavily for skin, and its intrinsic chemistry justifies some of that enthusiasm — but our data, together with the older photochemistry literature, suggest that unless it is formulated to shield it from light, much of its promise will be lost exactly when it is needed [23,30]. Photostable alternatives such as rutin, or strategies that physically protect the labile compound (encapsulation, for instance), look like the more rational route. This connects to a broader point that we think the field sometimes underweights: a photoprotective antioxidant has a dual job, and the second job — surviving the light — is easy to overlook when screening is done in the dark. It also has to be said that intracellular efficacy in a monolayer is not the same thing as efficacy in skin: quercetin, and especially its polar glycoside rutin, penetrate the stratum corneum poorly, so realistic topical use will probably depend on enabling formulations — nanocarriers, prodrugs or penetration enhancers — at least as much as on the intrinsic activity reported here.

V. CONCLUSION

The photostable polyphenols quercetin, rutin and silibinin significantly better restored mitochondrial bioenergetics (membrane potential, oxygen consumption, ATP and biogenesis markers) in primary human dermal fibroblasts exposed to an acute, sub-lethal UVB dose than the photolabile resveratrol. The ranking of efficacy followed the ranking of photostability rather than any obvious difference in baseline antioxidant strength, which — together with the equimolar and pre-irradiation control experiments — leads us to conclude that resistance to photodegradation is a key, though probably not the sole, requirement for a polyphenol meant to act under ultraviolet light. The most photostable of the set, rutin, gave the most complete recovery and is a good option for continued development on the basis of this evidence. Future work should progress to chronic exposure models, three-dimensional or ex vivo skin, and formulation experiments to evaluate whether these advantages withstand practical administration.

- *Conflict of Interest*

  The author declares no conflict of interest.

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- *Supplementary Materials*

The following supporting information is provided in a separate file (Supplementary Materials), and is referred to throughout the main text by its S-numbers:

Figure S1, UVB dose–response and dose selection; Figure S2, cytotoxicity of the polyphenols alone; Figure S3, PGC-1α and TFAM densitometry; Figure S4, representative HPLC chromatograms; Figure S5, equimolar (25 μM) comparison of viability and ΔΨm; Figure S6, confirmation of ΔΨm by TMRE; Figure S7, mitochondrial superoxide by MitoSOX; Figure S8, mitochondrial mass and citrate synthase activity. Table S1, dose–response data; Table S2, cytotoxicity data; Table S3, qPCR primer sequences; Table S4, antibodies for Western blotting; Table S5, HPLC conditions; Table S6, densitometry values; Table S7, confirmation in additional donor lots; Table S8, equimolar comparison data; Table S9, mitochondrial mass and citrate synthase data; Table S10, intrinsic (cell-free) antioxidant capacity of each compound (DPPH IC50 and Trolox-equivalent values).

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