# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente Ciclo XVIII

# Feeding skin cellular regeneration: the importance of 1C metabolism and the potential of 5-MTHF to counteract UV-induced damage

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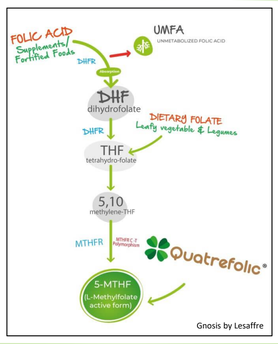
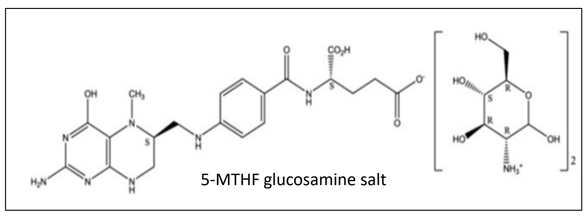
## Introduction

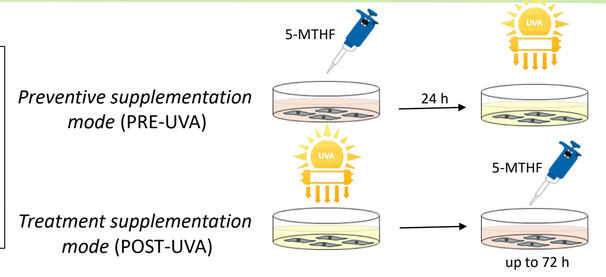
One-carbon metabolism, involving folate intermediates like 5-methyltetrahydrofolate (5-MTHF), is vital for DNA synthesis, epigenetic regulation, and cell repair. In the skin, 5-MTHF supports fibroblast activity, collagen synthesis, and regeneration, but solar radiation - especially in aging skin - reduces folate levels, impairing these processes and increasing deficiency risk [1]. While folate's nutritional role is well-known, its skincare potential as a regenerative and anti-photoaging agent remains underexplored. Most research has focused on folic acid [2,3], a synthetic form needing intracellular activation and posing formulation issues due to instability and poor solubility. In contrast, new, reduced folate salts are bioequivalent to active folate and offer better stability, penetration, and antioxidant effects, making them promising for cosmetic and therapeutic use [4,5].




## Aim and Experimental design

The objective of this study was to evaluate the modulation by 5-MTHF of UV-induced damage in skin cells. The study was conducted in-vitro on human dermal fibroblasts (HDF) where we aimed to test the potential beneficial effects of 5-MTHF on skin health, in particular on its regenerative capacity using wound healing cellular models.




## Results

### Bioavailability and uptake of 5-MTHF

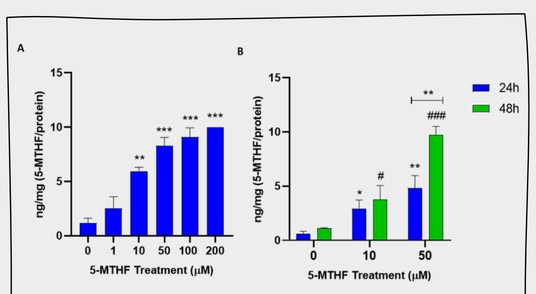



**Figure 1.** *5-MTHF intracellular content in HDF, analyzed by HPLC.* (A) Intracellular content detected 24 h after treatment with increasing concentrations of 5-MTHF. (B) Intracellular content detected 24 h and 48 h after treatment with 10 μM and 50 μM 5-MTHF. Statistical analysis was performed using Ordinary one-way and 2-way ANOVA (Sidak's and Tukey's multiple comparisons tests were used). * p < 0.05, ** p < 0.01, *** p < 0.0001 vs 0 (24 h); # p < 0.05, ### p < 0.0001 vs 0 (48 h).

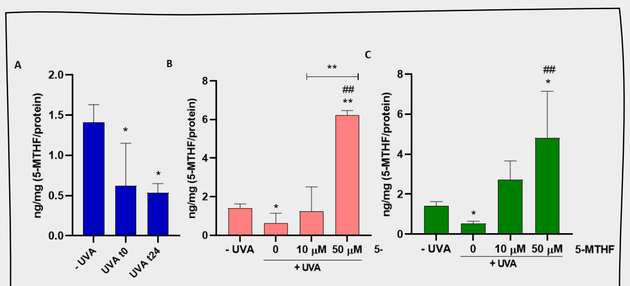



**Figure 2.** *5-MTHF intracellular content in HDF, analyzed by HPLC.* (A) Levels measured immediately (0 h) and 24 h after 10 min UVA exposure (~270 kJ/m²). (B) Intracellular content detected in cells pre-treated for for 24 h with 10 μM and 50 μM 5-MTHF and then exposed to 10 min UVA . (C) Intracellular content in cells exposed to 10 min UVA and then treated for 24 h with 10 μM and 50 μM 5-MTHF. Statistical analysis was performed using Ordinary one-way ANOVA (Sidak's multiple comparisons test). * p < 0.05, ** p < 0.01 vs -UVA; ## p < 0.01 vs 0.

### Cytosolic and mithocondrial ROS production

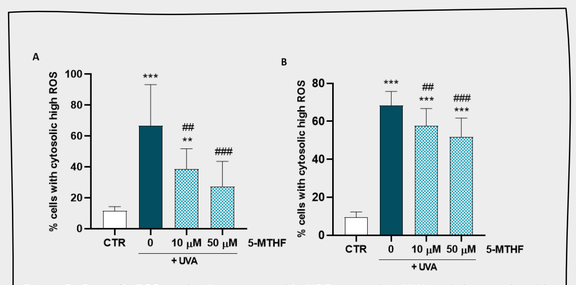



**Figure 3.** *Cytosolic ROS production measured in HDF exposed to UVA and then analyzed by flow cytometry.* (A) Cells were exposed to 10 min UVA, then 10 μM and 50 μM 5-MTHF were added straight after, and the percentage of cells with high cytosolic ROS analyzed 24 h later. (B) Cells were treated for 24 h with 10 μM and 50 μM 5-MTHF and then exposed to 10 min UVA. The percentage of cytosolic high ROS were then analyzed straight after. CTR=non-irradiated control. Statistical analysis was performed using Ordinary one-way ANOVA (Sidak's multiple comparisons test). ** p < 0.01, *** p < 0.0001 vs CTR; # p < 0.05, ## p < 0.01, ### p < 0.0001 vs 0.

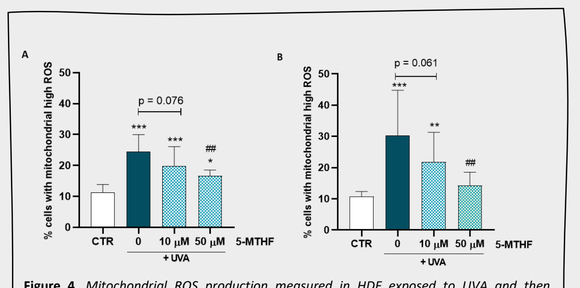



**Figure 4**. *Mitochondrial ROS production measured in HDF exposed to UVA and then analyzed by flow cytometry.* (A) Cells were exposed to 10 min UVA, then 10 μM and 50 μM 5-MTHF were added straight after, and the percentage of cells with high mitochondrial ROS analyzed 24 h later. (B) Cells were treated for 24 h with 10 μM and 50 μM 5-MTHF and then exposed to 10 min UVA. The percentage of mitochondrial high ROS were then analyzed straight after. CTR=non-irradiated control. Statistical analysis was performed using Ordinary one-way ANOVA (Sidak's multiple comparisons test). * p < 0.05, ** p < 0.01, *** p < 0.0001 vs CTR; ## p < 0.01 vs 0.

### Wound healing

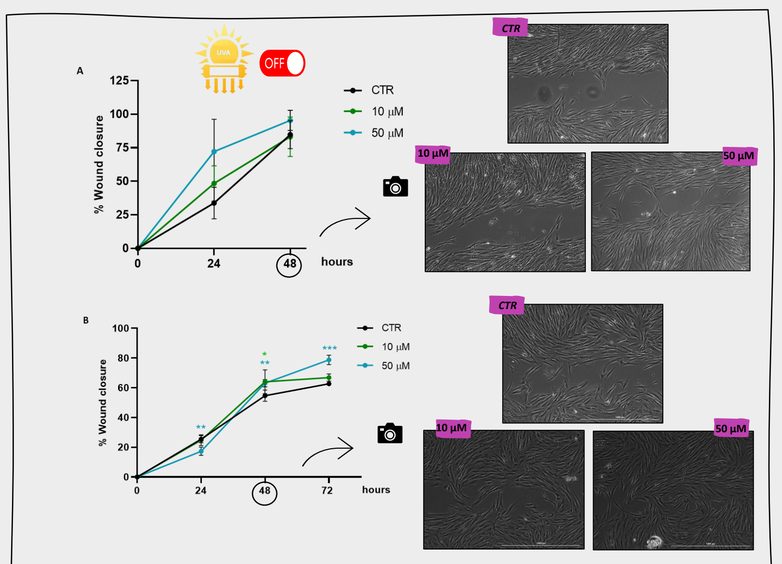



**Figure 5.** *Wound healing measured as percentage of wound closure in HDF analyzed by ImageJ software using the Wound Healing size tool.* (A) After the wound was performed, 10 μM and 50 μM 5-MTHF were added and wound closure was monitored for up to 48 h. (B) Cells were exposed to 10 μM and 50 μM 5-MTHF for 24 h before wound was performed. Wound closure was then monitored for up to 72h. CTR=cells without 5-MTHF. Statistical analysis was performed using Mixed-effects analysis (Sidak's multiple comparisons test). * p < 0.05 , ** p < 0.01, *** p < 0.0001 vs CTR.

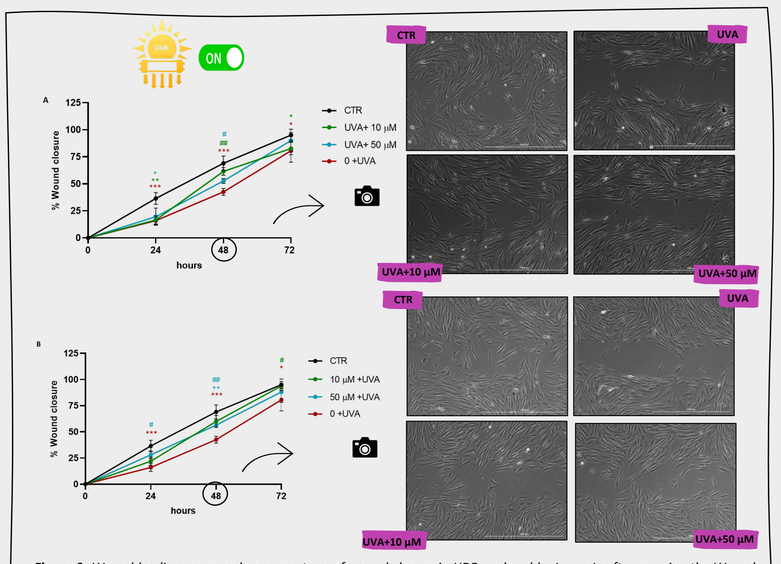



**Figure 6.** *Wound healing measured as percentage of wound closure in HDF analyzed by ImageJ software using the Wound Healing size tool.* (A) After the wound was performed, cells were exposed to 3 min UVA (~81 kJ/m²) and then 10 μM and 50 μM 5-MTHF were added. (B) Cells were exposed to 10 μM and 50 μM 5-MTHF for 24 h before wound was performed and then were exposed to 3 min UVA. Wound closure was monitored for up to 72h. CTR=cells without 5-MTHF. Statistical analysis was performed using Mixed-effects analysis (Sidak's multiple comparisons test). # p < 0.05, ## p < 0.01 vs 0 + UVA; * p < 0.05 ,** p < 0.01, *** p < 0.0001 vs CTR

### DNA damage

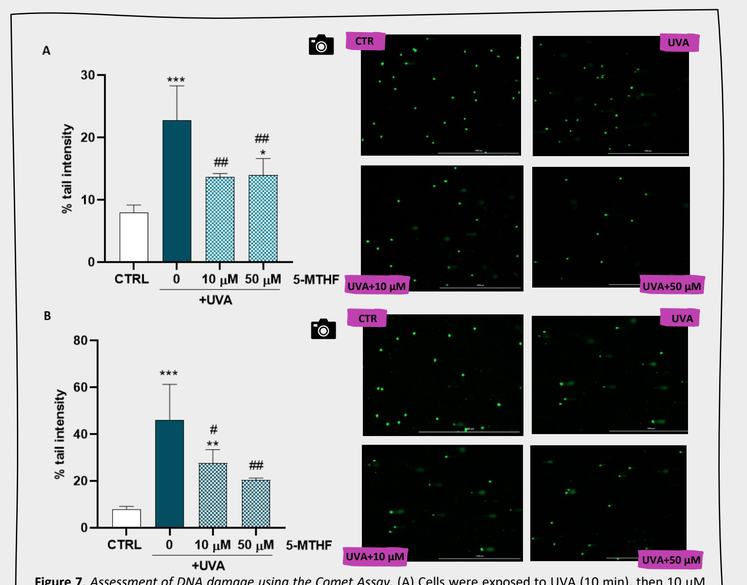



**Figure 7**. *Assessment of DNA damage using the Comet Assay.* (A) Cells were exposed to UVA (10 min), then 10 μM and 50 μM 5-MTHF were added straight after and kept for 24h. (B) Cells were treated for 24 h with 10 μM and 50 μM 5-MTHF, then exposed to 10 min UVA. CTR=non-irradiated control. The graph shows the extent of DNA strand breaks as measured by the percentage of the tail intensity. Statistical analysis was performed using Ordinary one-way ANOVA (Sidak's multiple comparisons test). * p < 0.05, ** p < 0.01, *** p < 0.0001 vs CTR; # p < 0.05, ## p < 0.01 vs 0.

## Conclusions

Supplementation with 5-MTHF shows dose-dependent uptake by HDFs, remaining stable for up to 48 hours. Additionally, 5-MTHF provides significant protection against UVA-induced oxidative stress, reducing cytosolic and mitochondrial ROS production, especially at higher concentrations. This antioxidant effect improves cell viability after UVA exposure. 5-MTHF also enhances wound healing, as shown by improved closure rates in scratch assays. The photoprotective effect of 5-MTHF, both before and after irradiation, was confirmed through the comet assay, demonstrating reduced DNA damage at all tested concentrations. The impact of 5-MTHF in the remodelling of dermal structural components is also being studied. Overall, the research points to 5-MTHF's potential as a therapeutic agent for promoting skin health, protecting against oxidative damage, and enhancing wound healing, although further investigation is required to confirm these outcomes in clinical settings.

## References

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