

Effect of Blue light on *ex vivo* human skin. Evaluation of the activity of L-Carnosine.

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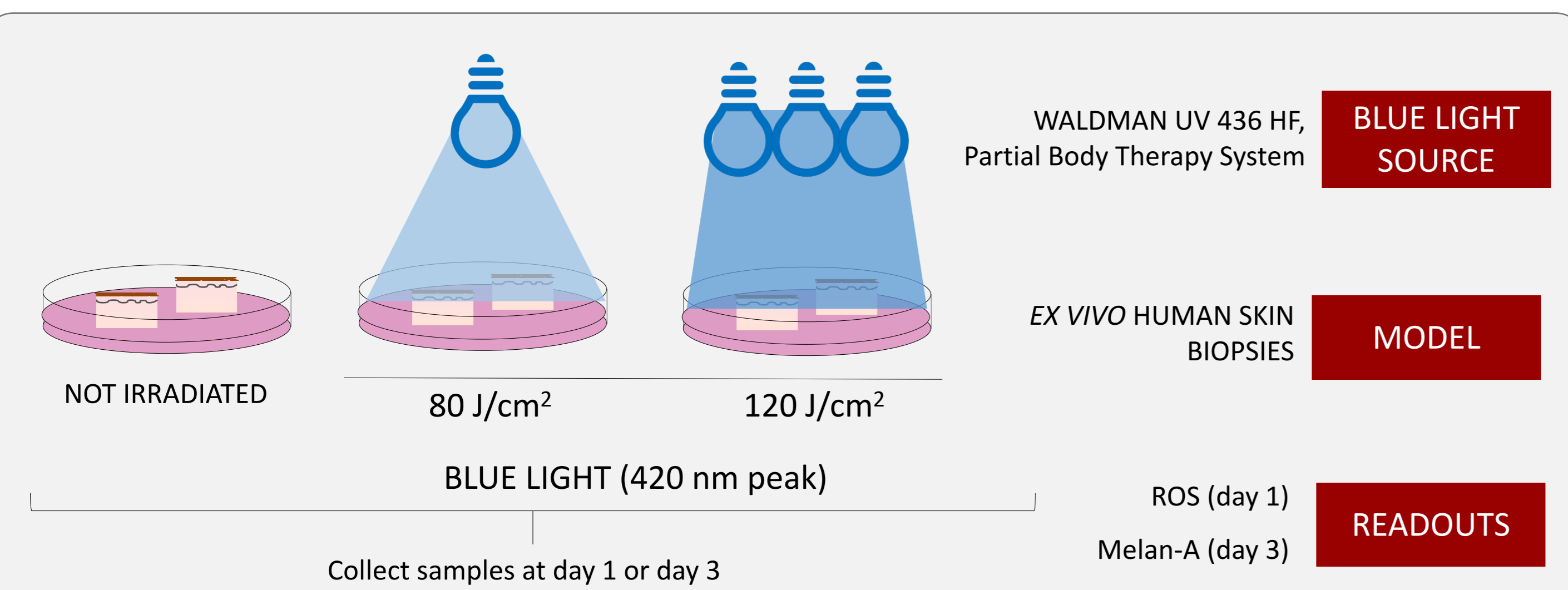
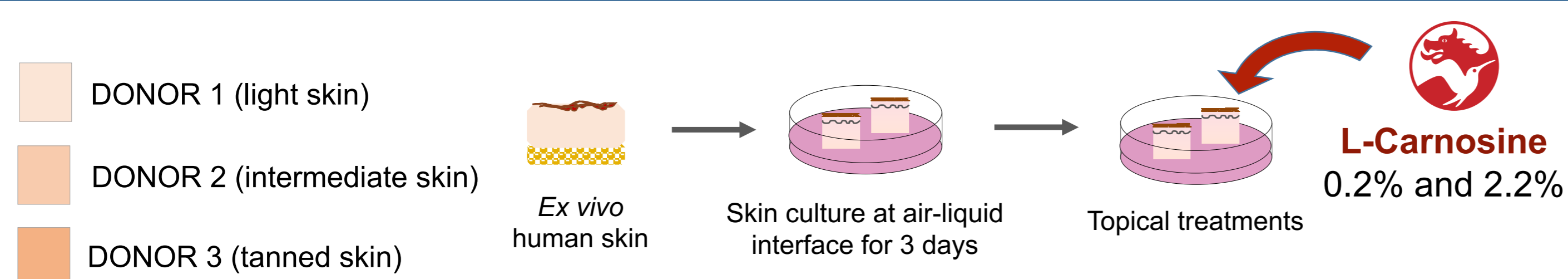
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Introduction:



The main function of the skin is to protect the body against negative environmental influence such as light radiation. UV light is well known to cause a variety of effects [1–3], but recently it has been shown that the high energy part of visible spectrum “Blue light” (400-500 nm) (BL) deeply penetrates into the skin, generating reactive oxygen species (ROS), oxidative damage and inducing melanogenesis [4–6]. The main source of BL is the sunlight, but there are also artificial sources of BL (cell phones, monitors, light-emitting diodes), thus the demand for cosmetic products claiming BL protection is increasing [7]. The aim of this work is to investigate the effects of BL using an *ex vivo* skin model and test the efficacy of L-Carnosine on protecting skin from BL effects.

Materials & Methods:

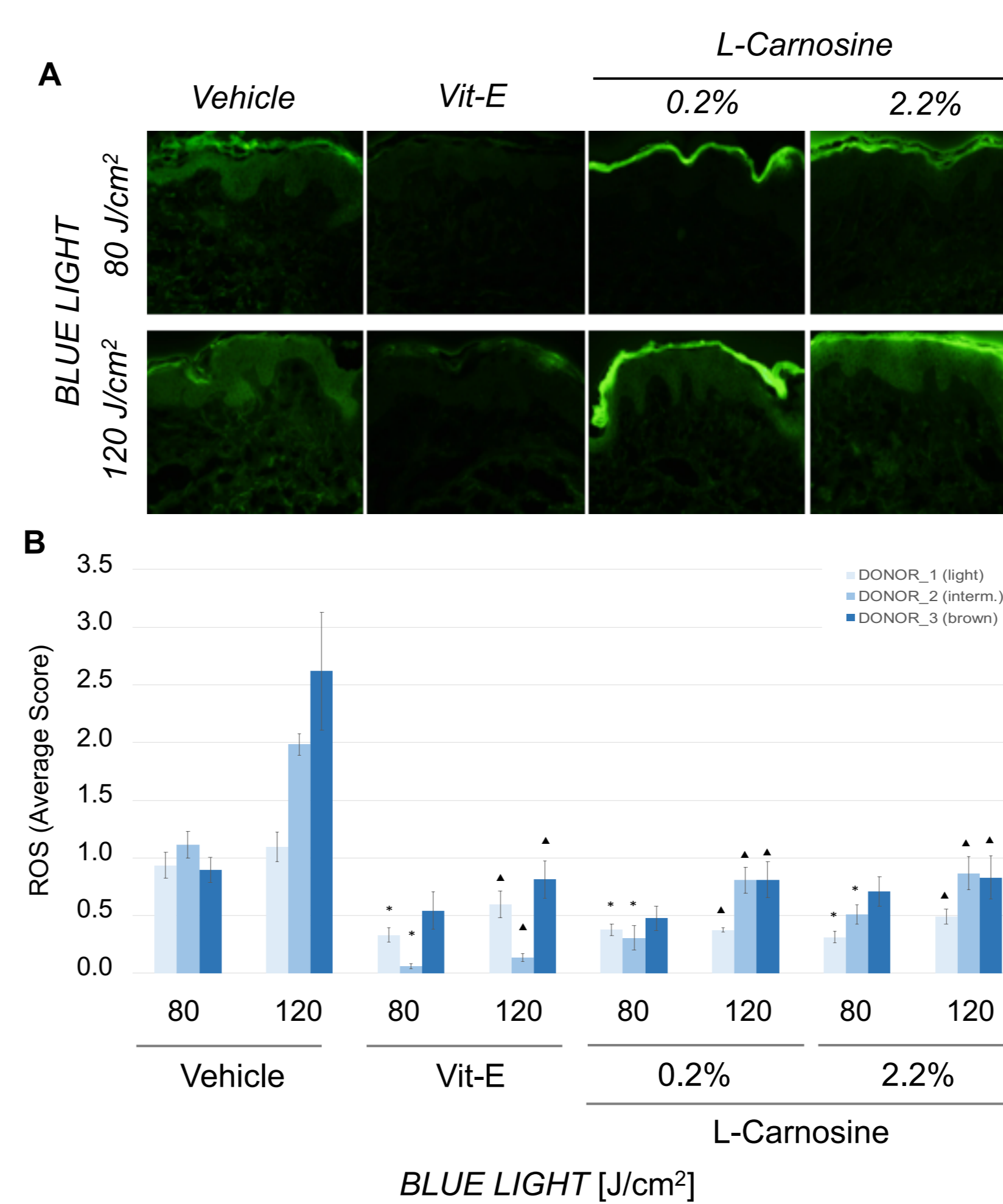


- **ROS evaluation (day1):** Dichlorofluoresceine diacetate (DCFH-DA) method. Before irradiation, skin samples were treated overnight with vehicle or test compounds (L-Carnosine). The amount of ROS was evaluated semi-quantitatively measuring the fluorescent signal in the dermal part of the samples by image analysis.
- **Skin pigmentation (day3):** Immuno-staining with Melan-A antibody. The full length of the tissue samples was evaluated for the presence of Melan-A positive cells and their number was normalized on the basal lamina length.

Results & Discussion:

Blue light induces oxidative stress and stimulates melanogenesis

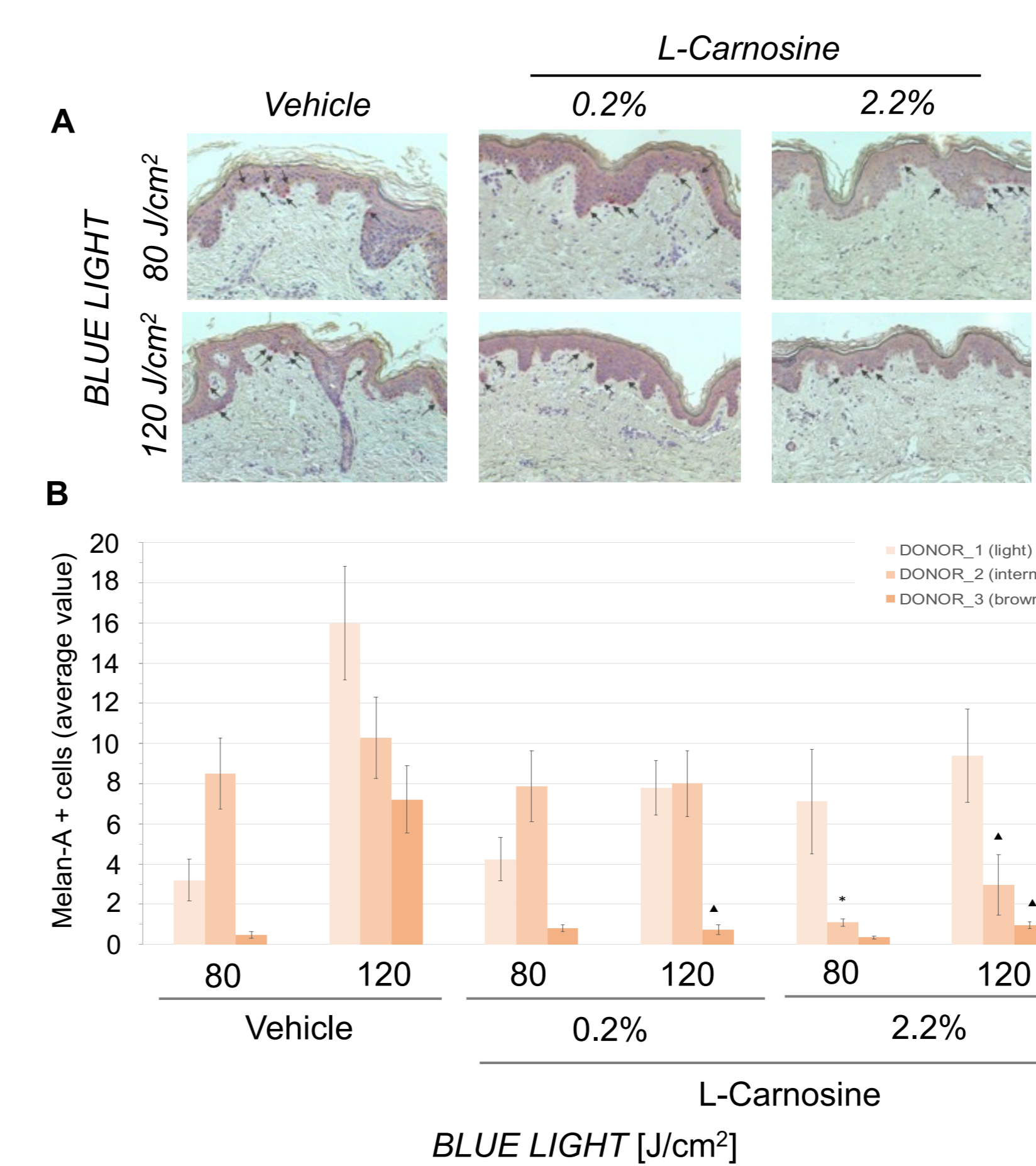
- Both doses of BL significantly induced oxidative stress in light and intermediate skin (donor 1 and 2), whereas in the tanned phototype (donor 3) only 120 J/cm² of BL significantly increased the level of ROS (data not shown).
- A significant increase of Melan-A positive cells was observed with both irradiation doses in intermediate skin (donor 2), whereas in light and tanned phototypes (donor 1 and 3 respectively) a significant increase of Melan-A positive cells was found only after 120 J/cm² of BL (data not shown).



L-Carnosine significantly reduces ROS

- Results showed a significant decrease of ROS production in all skin phototypes treated with L-Carnosine.
- We found an average ROS modulation of -60% and -47% after 80 J/cm² and of -65% and -60% after 120 J/cm² in samples treated with 0.2% and 2.2% of L-Carnosine.
- The positive antioxidant control (VIT-E) confirmed the anti-oxidant effect of L-Carnosine in our model.

Fig. 1. Decrease of ROS production in L-Carnosine treated samples. ROS production was assessed by DCFH-DA assay; fluorescence was evaluated in the upper dermis. A) Representative images. B) Graph showing ROS level (mean score) in treated and control samples. Error bars indicate the standard error of mean (SEM). * Significantly different from 80 J/cm² irradiated control, ▲ significantly different from 120 J/cm² irradiated control (Tukey's test, p<0.05).



L-Carnosine reduces the number of Melan-A positive cells

- In intermediate skin (donor 2), L-Carnosine 2.2% significantly reduced Melan-A positive cells both after 80 J/cm² (-87%) and 120 J/cm² (-71%) of BL irradiation.
- In tanned skin (donor 3) exposed to 120 J/cm² of BL, L-Carnosine at 0.2% and 2.2% significantly reduced the amount of Melan-A positive cells by -90% and -87%, respectively.

Fig. 2. Decrease of Melan-A positive cells in L-Carnosine treated samples. Melan-A protein level was assessed by immunohistochemical staining. A) Representative images. B) Graph showing Melan-A level (mean value). Error bars indicate the standard error of mean (SEM). * Significantly different from 80 J/cm² irradiated control, ▲ significantly different from 120 J/cm² irradiated control (Tukey's test, p<0.05).

Conclusions:

- Blue light exerts a pro-oxidative effect on the skin and leads to an increase in Melan-A-positive cells.
- Our *ex vivo* human skin model is a valuable system to assess the consequences of blue light radiation and the capacity of applied compounds to counteract them.

L-Carnosine represents an effective and reliable solution to mitigate the adverse effects of BL radiation on the skin reducing both ROS levels and Melan-A positive cells.

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