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Beneficial Effect of Dietary Epigallocatechin-3-Gallate on Skin via Enhancement of Antioxidant Capacity in Both Blood and Skin

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Key Words
Dietary antioxidant · UVB irradiation · Skin · Blood · Total antioxidant capacity

Abstract
Background/Aims: Dietary antioxidants exert a photoprotective effect against UV radiation. However, the mechanism underlying the beneficial effects on skin of orally administered antioxidant is not very clear. The present study aimed to investigate the UVB-induced total antioxidant capacity (TAC) perturbation both in skin and blood and to study the beneficial mechanism of action of dietary epigallocatechin-3-gallate (EGCG). ***Method:*** Female HWY/Slc hairless rats were exposed to UVB radiation for 4 weeks and skin and blood TAC were measured at weeks 0, 2, and 4. The correlation between skin and blood TAC was assessed using Pearson's correlation analysis. To investigate the effect of dietary EGCG, female HWY/Slc hairless rats were fed EGCG and exposed to UVB radiation. At the end of the 8-week experimental period, transepidermal water loss (TEWL) and epidermal thickness were measured to assess skin damage, and TAC was analyzed in both skin and blood. ***Results:*** UVB radiation significantly decreased skin and blood TAC, and there was a significant correlation between skin and blood TAC. Dietary EGCG significantly prevented UVB-induced adverse effects on epidermal thickness and TEWL, and the UVB-induced decrease in TAC recovered in both skin and blood. ***Conclusion:*** These results demonstrate that there is a close relationship between skin and blood TAC and the beneficial effect of oral antioxidants on skin may possibly be mediated through significant enhancement of blood TAC.

Introduction

Skin aging is influenced by several factors, including genetics, metabolic processes, hormonal changes, and environmental exposure. Because skin is the outermost barrier of the body, it is very susceptible to external disturbances and directly interacts with harmful environments, such as UV radiation, pollutants, and mechanical stress [1]. Oxidative stress following UV radiation is thought to play a central role in initiating and driving the signaling events that lead to skin aging [2]. It has been demonstrated that treatment with antioxidants can prevent the deleterious effect of oxidative stress caused by UV radiation, thus providing a photo-protective effect against UV-induced skin damage.

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The classical route of antioxidant administration to the skin is topical application. The primary action of topically applied antioxidants is to quench the free radicals produced by UV radiation. For this route to be effective, the bioactive compound must be stable throughout its preparation and should be able to penetrate the skin. Additionally, the penetration of antioxidants into the skin is influenced by environmental factors, such as temperature and hydration [3]. Considering these topical application limitations, there has been increasing interest in oral consumption as an alternative route for antioxidant supplementation to prevent skin aging. Several clinical trials have demonstrated that oral antioxidants, such as vitamin C, vitamin E, and carotenoids, can prevent deleterious effects related to oxidative stress and skin aging [4–6]. Although it has been demonstrated that dietary antioxidants have beneficial effects on skin, the precise mechanism of action is still unknown.

It is controversial whether oral antioxidants, especially hydrophilic bioactive compounds such as polyphenols, are delivered to skin tissue. Oral consumption is primarily distinguished from topical application by the fact that it requires systemic distribution. Oral consumption could have beneficial effects directly by acting on skin tissue or indirectly via a systemic effect on the vascular system. It is well known that blood affects the condition of the skin by supplying the cells and tissues with nutrients and bioactive compounds [7, 8]. We should clarify the complex interplay between blood and skin in order to study the mechanism underlying the beneficial effects of oral consumption.

The objective of this study was to investigate the effect of an external stressor, UV radiation, on the total antioxidant capacity (TAC) in both skin and blood to understand the relationship of skin and blood TAC. Oxidative stress-related changes following UV radiation have been studied in the skin of humans and hairless mice and erythrocytes of Wistar rats [9, 10]. However, physiological data regarding the relationship between skin and blood TAC changes following UV exposure is scarce. The hairless rat, used in this study, appears to be a useful model for studying oxidative stress-related mechanisms after UV radiation, simultaneously in skin and blood [11, 12]. We also evaluated the effect of dietary epigallocatechin-3-gallate (EGCG) on UVB-induced TAC perturbations in skin and blood, as well as UVB-induced skin damage. EGCG, a major polyphenolic constituent of green tea, is a very potent antioxidant that counteracts the deleterious effects induced by oxidative stress. It has recently been shown that oral administration of green tea polyphenols in drinking water can prevent the UV-induced cutaneous edema and decrease markers of cutaneous photoaging, such as MMPs [13, 14].

There is no previous report investigating the relationship of skin and blood TAC to understand the mechanism of the beneficial effects of oral consumption. Furthermore, to our knowledge, this is the first reported study that simultaneously examined skin and blood TAC changes after oral consumption of an antioxidant in an animal model.

Materials and Methods

Animals

Female HWY/Slc hairless rats (9 weeks old, weighing 190–210 g) were obtained from SLC Inc. (Shizuoka, Japan). HWY/Slc rats are derived from a hairless mutant in a colony of rats of the Wistar strain and have been maintained as an inbred strain by SLC [15]. The animals were acclimatized for 1 week in the animal facility prior to the experiments and were housed under controlled temperature (23 ± 2°C), humidity (55 ± 10%), and light (12/12 h light/dark, with no ultraviolet emission). All care and treatment of the animals and husbandry were carried out in compliance with and were approved by the institutional animal care and use committee of the Amorepacific R&D center.

Reagents

EGCG (>94%) was obtained from DSM Ltd. (formerly F. Hoffmann-La Roche, Basel, Switzerland). All other chemicals were of analytical or HPLC grade.

Experimental Design

To investigate the effect of UV irradiation on the TAC in skin and blood, 30 female HWY/Slc hairless rats were randomly divided into 2 groups, the non-irradiated control (control) and UV irradiated (UV-stress) groups. Each group was then subdivided into 3 groups to collect blood and skin samples at different time points: 0, 2, and 4 weeks of UV irradiation. Each subgroup of 5 rats was housed in 1 cage.

To evaluate the effect of dietary EGCG on UV-induced TAC alteration in skin and blood, 50 female HWY/Slc hairless rats were randomly divided into 3 groups, non-irradiated control (control), UV irradiated control (UV-control), and UV irradiated-EGCG-treated (UV+EGCG) groups. Each group of 5 rats was housed in 1 cage. Skin and blood samples were collected at the end of the 8-week experiment.

All the rats, except for the EGCG-treated group, were fed a commercial rat food that contained protein (~20%) and fat (~7%). The EGCG-treated group was fed the normal diet supplemented with 1,500 ppm EGCG (Feedlab Korea Corp., Seoul, Korea). Food and water were provided ad libitum. Food consumption and body weight were recorded during the experimental period.

UV Irradiation

The rats were irradiated 3 times a week at a dose of 97 mJ/cm^2 (equivalent to 1 MED) to induce oxidative stress, using an array of seven UV-B lamps surrounding three UV-A lamps (Waldmann UV800, Villingen-Schwenningen, Germany; 285–350 nm, peak

at 310–315 nm) [16]. The integrated UV irradiance was measured with a Waldmann UV meter (Waldmann Lichttechnik GmbH).

Sample Preparation

Blood samples were collected from the orbital sinus in heparinized tubes and centrifuged (3,000 *g*, 10 min) for plasma separation to measure TAC. Biopsies, obtained from the central dorsal skin, were cleaned of the adipose tissue and a skin homogenate (25%, w/w) was prepared from full-thickness skin using a PRO 200 homogenizer (PRO Scientific Inc., Monroe, Conn., USA) for biochemical determinations. The homogenate was centrifuged (15,000 *g*, 5 min) and the supernatant was used in the analysis. Proteins were quantified with a BCA™ Protein Assay (PIERCE, Rockford, Ill., USA) before the skin's antioxidant status was analyzed. The samples were stored at –70°C until used.

TAC Analysis

The TAC of the plasma and skin homogenates were measured as Trolox equivalent antioxidant capacities (TEAC), according to Miller et al. [17, 18]. ABTS (2,2′-azino-di-2-ethylbenzthiazoline sulfonate) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$. This had a relatively stable blue-green color, which was measured at 405 nm. Antioxidants in the sample caused the suppression of this color production to a degree proportional to their concentration. The results were expressed as mmol TEAC/l of plasma or mmol TEAC/mg of protein.

Assessing Transepidermal Water Loss

Transepidermal water loss (TEWL) was measured 48 h after the final irradiation to allow recovery from any acute UV effect. We determined the TEWL as a marker of epidermal barrier function using a wireless vapometer (Delfin Technologies Ltd., Kuopio, Finland). All measurements were carried out at 23 ± 2°C and a humidity level of 50 ± 10% and were performed in duplicate for each skin spot.

Histological Evaluation

At 48 h after the last irradiation, the rats were sacrificed and biopsies were obtained from the central dorsal skin, perpendicular to the long axis of the trunk. The biopsies were fixed in 10% buffered formalin and prepared for optical microscopy. Masson-Trichrome staining was conducted for a routine examination of the tissue and to measure the epidermal thickness. Quantitative analysis of the histology sections was carried out using 2 contiguous histological sections, which were each photographed at the midpoint of the section (Masson-Trichrome stain, ×100 magnification). The thickness of the epidermis was evaluated by taking 10 representative sample measurements per tissue section on the microscope.

Statistics

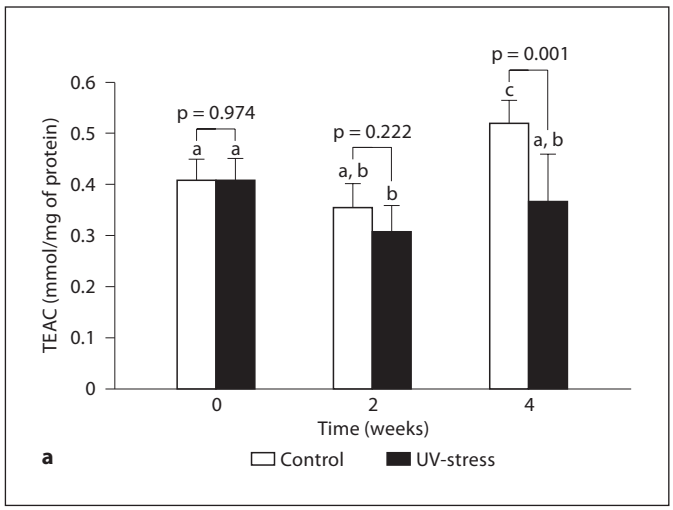
Results are expressed as means ± SD. Statistical analysis was performed using the SPSS software (SPSS 12.0). A one-way ANOVA with Duncan's LSD test was used to compare mean values. Pearson's correlation test was used to analyze a possible relationship between variables using MINITAB 14 (Minitab Inc., State College, Pa., USA). Values of $p < 0.05$ were deemed to indicate statistical significance.

Results and Discussion

Effect of External Stressor (UV Irradiation) on the TAC in Skin and Blood

It was previously reported that acute and chronic UV irradiation significantly altered enzymatic and non-enzymatic antioxidant systems in the blood and epidermis of hairless rats [19]. In the present study, we observed UV-induced TAC changes in blood and skin throughout the 4-week study period, and the results are shown in figure 1. The initial TEAC values of both blood and skin samples showed no significant difference between the control and UV-stress groups. We unexpectedly observed significant changes in skin and blood TEAC values in the controls throughout the experiment period. After 2 weeks of the experiment, the skin and blood TEAC values of the controls were markedly decreased as compared to the initial value, from 0.41 ± 0.04 to 0.35 ± 0.05 mmol/mg of protein and from 1.42 ± 0.18 to 0.95 ± 0.11 mmol/l of plasma, respectively. The skin and blood TEAC values increased again, to 0.35 ± 0.05 mmol/mg of protein and 0.95 ± 0.11 mmol/l of plasma, respectively, after the 4 weeks of the experiment. Plasma and tissue antioxidant concentrations depend on the state of health, diet, skin type, and external factors [19]. It has been reported that TAC is influenced by several factors, such as age, gender, life style, and diet [20, 21]. The animals used in this study were of the same strain, gender, and age, and were housed under the same controlled conditions. Moreover, no other side effect was detected throughout the experiment. Thus, we believe that the TEAC value changes in the controls are normal variation, rather than experimental error. However, it is hard to explain what might cause the changes.

At 2 and 4 weeks of exposure to UVB radiation, there were approximately 14 and 30% decreases in skin TEAC values versus those of matched controls, but only at 4 weeks of exposure to UVB radiation was the difference statistically significant. Analysis of TEAC values in blood samples showed significant decreases in TEAC values after 2 and 4 weeks of UVB radiation in comparison with matched controls, i.e. 43 and 44% decreases, respectively, versus the matched controls. Because skin is directly exposed to UV radiation, the changes in skin TAC were expected to be greater than those in blood TAC. However, blood TEAC values displayed markedly larger decreases with UVB irradiation than skin TEAC. The blood is known to continuously replenish the skin with nutrients, bioactive compounds, cytokines, and enzymes to cope with stressors. The blood may absorb UVB-induced neg-



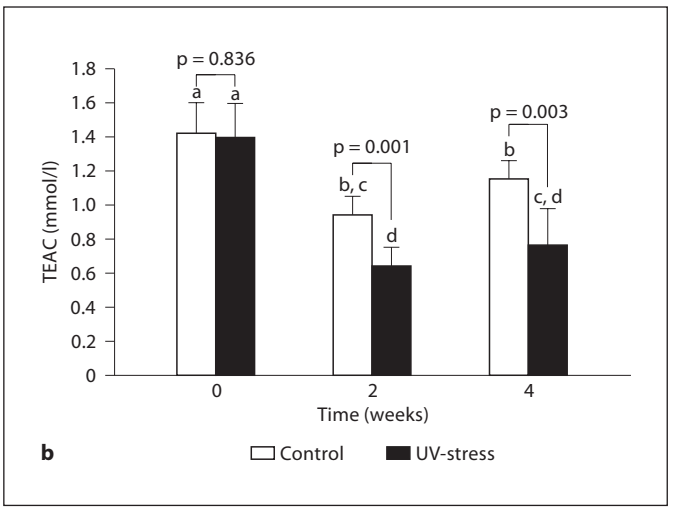


Fig. 1. Effect of UV irradiation on TAC in skin (**a**) and blood (**b**). In each group of rats, blood samples were collected at the indicated times from the orbital sinus, in heparinized tubes, and skin biopsies were obtained from central dorsal skin to measure TAC. Proteins were quantified before analytical processing. Data represent means ± SD. Within each graph, values with different superscript letters (a–d) are significantly different ($p < 0.05$).

ative alterations in skin tissue and supplement the antioxidants in the skin, resulting in greater alterations in blood TEAC values.

The results of Pearson's correlation analysis indicated a statistically significant linear relationship between the skin and blood TEAC values ($r = 0.715$; $p < 0.001$). This suggests that the external stressor significantly affects blood conditions and that there is a close relationship between skin and blood TAC. While the TEAC values cannot provide information on the full complement of antioxidants present, they are useful for determining overall antioxidant levels in plasma and tissue homogenates [22].

Effect of Dietary EGCG on UV-Induced Skin Damage and TAC Alterations in Skin and Blood

Since the plasma and tissue antioxidant capacity varies among individuals and is influenced by the health status, diet, external factors, and different skin types, we randomly assessed initial plasma TEAC values in subgroups of each group. The values were not significantly different (data not shown). The 8-week UVB irradiation period significantly decreased the TEAC values in skin homogenates from 0.24 ± 0.03 to 0.19 ± 0.04 mmol/mg of protein as compared to the control, and dietary EGCG significantly prevented the decrease (fig. 2a). UVB radiation reduced the plasma TEAC values from 1.44 ± 0.06 to 1.31 ± 0.07 mmol/l of plasma, and EGCG significantly reduced the UVB-induced decrease in TEAC value to 1.50 ± 0.04 mmol/l of plasma (fig. 2b). Remarkably, the difference between the control and UV-control was smaller in experiment 2 after 8 weeks of UVB radiation than in experiment 1 after 2 and 4 weeks of UVB radiation. Chronic exposure to mild oxidative stress is known to increase cellular resistance to the toxic effect of exogenous or endogenous ROS exposure [23]. Such adaptation was associated with both high glutathione content and over-expression of a large set of genes encoding antioxidant proteins [24]. During the 8-week experimental period, the rats may acquire tolerance to oxidative stress with time.

At the end of the 8-week oral administration of EGCG, we assessed epidermal thickness and TEWL as UVB-induced skin damage parameters. UV irradiation is known to disturb the highly organized stratum corneum, and thus increase the TEWL by decreasing skin barrier integrity. It also induces a progressive thickening of the epidermis [25]. The 8 weeks of UVB radiation markedly increased TEWL to 16.0 ± 2.1 $g/m^2/h$ when compared with the control, 13.5 ± 1.4 $g/m^2/h$; however, the UVB-induced increase in TEWL was significantly decreased to 11.0 ± 1.2 $g/m^2/h$ by EGCG (fig. 3a). Histological observation indicated that the epidermal thickness in the irradiated group was significantly increased as compared to the control, and that the increased thickness was significantly reduced by EGCG. The mean epidermal thickness was 34.9 ± 1.2, 42.9 ± 1.8, and 38.9 ± 1.2 μm in the control, UV-control, and UV+EGCG groups, respectively (fig. 3b).

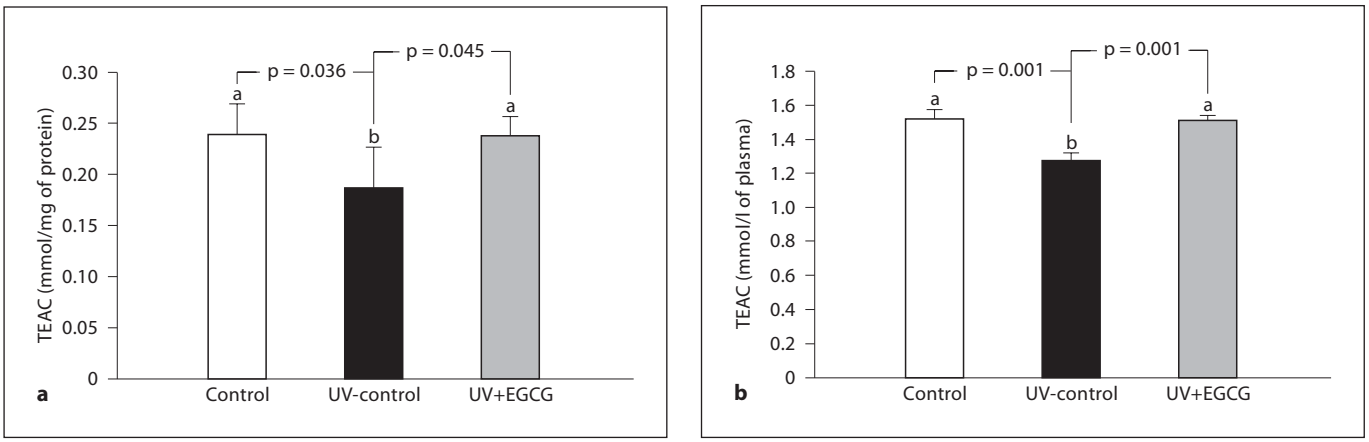


Fig. 2. Effect of dietary EGCG on UV-induced TAC alterations in skin (**a**) and blood (**b**). At the end of an 8-week oral administration of EGCG, blood samples were collected from the orbital sinus in heparinized tubes and centrifuged for plasma separation to measure TAC. Skin biopsies were obtained from central dorsal skin and skin homogenates were prepared to measure TAC. Proteins were quantified before analytical processing. Data represent means ± SD. Within each graph, values with different superscript letters (a, b) are significantly different ($p < 0.05$).

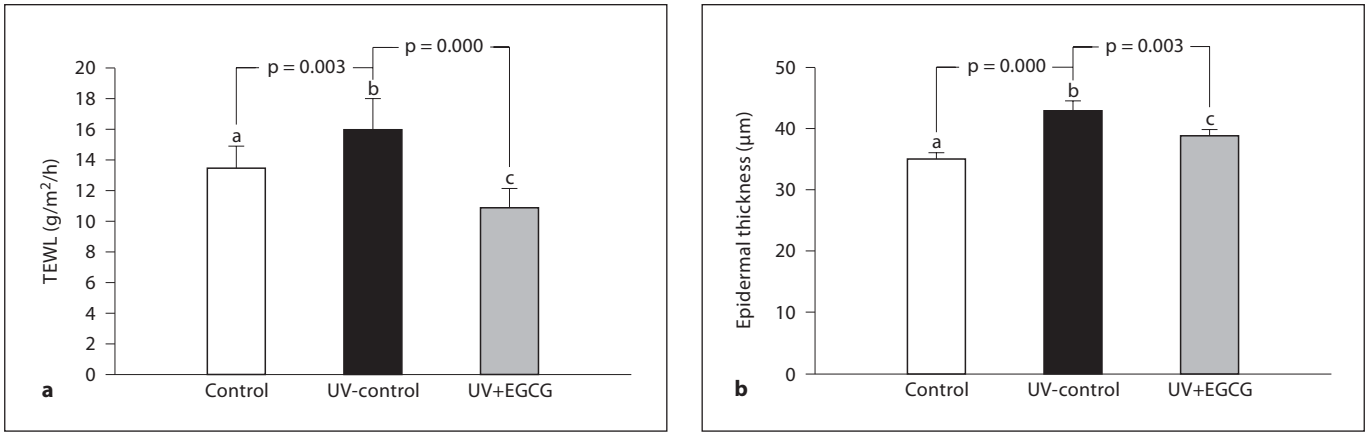


Fig. 3. Effect of dietary EGCG on the UVB-induced skin barrier function perturbation and epidermal thickening. At the end of an 8-week oral administration of EGCG, TEWL was measured (as a marker of the skin barrier function; **a**) and skin biopsies were obtained from central dorsal skin to measure epidermal thickness (**b**). Data represent means ± SD. Within each graph, values with different superscript letters (a–c) are significantly different ($p < 0.05$).

We observed a disturbance in skin and blood TAC by the external stressor, UVB radiation, and EGCG significantly reduced the disturbance and UVB-induced skin damage. Recently, it was demonstrated that EGCG directly inhibited the expression of MMPs, such as MMP-9, MMP-2, MT1-MMP and neutrophil elastase, at pharmacologically achievable concentrations [26, 27]. Because EGCG has a hydrophilic nature, the tissue distribution of EGCG is likely to be limited. Indeed, it is not obvious that EGCG is pharmacologically relevant in skin. Vayalil et al. [14] – who reported the inhibitory effect of dietary green tea polyphenols on UV-induced expression of MMP-2, MMP-3, MMP-7, and MMP-9 – assumed that dietary EGCG may not directly affect skin cells in vivo, but could have indirect effects through cells in other tissues. In the present study, we found that there was a strong correlation between skin and blood TAC. This finding provides evidence that the skin-protective effect of dietary EGCG in vivo in skin may be mediated by improving blood TAC, rather than directly acting on skin tissue. Bolster-

ing the antioxidant defense system of the skin is an important strategy for reducing environmentally induced skin damage [28]. Blood is a specialized body fluid that delivers necessary substances to the whole body's tissues and cells, such as nutrients and oxygen, and transports waste products away from those tissues and cells. Thus, blood TAC may affect skin TAC and alleviate the detrimental effects of UV radiation.

However, the antioxidant defense system of the organism is a complex network and comprises several antioxidants. Thus, the result in this case of the single compound does not represent the full underlying biochemical mechanisms of dietary antioxidants. Moreover, not only positive but also negative results have been observed with high doses of a single dietary antioxidant compound [29, 30]. Although the use of dietary supplement for photoprotection should be approached cautiously, there is convincing evidence that dietary antioxidants can provide protection against skin damage from UV. The results of this study support the idea that an indirect action, via a systemic effect via the vascular system, may be one of the beneficial mechanisms of action of a dietary antioxidant on skin.

Conclusions

In summary, skin and blood TAC were markedly changed by UVB radiation, and EGCG significantly reduced the disturbance and UVB-induced skin damage. Considering the low bioavailability and the hydrophilic nature of EGCG, EGCG is not thought to directly act on skin tissue. It may be that the beneficial effect of oral antioxidants on skin is mediated through a significant enhancement in blood TAC, which consequently improves the skin TAC and alleviates the UV-induced TAC changes. However, this result does not prove a causal relationship between skin and blood TAC. The mechanism by which dietary antioxidants affect UV-induced responses in the skin needs to be studied further. To examine the close relationship between blood and skin TAC, in vivo tissue distribution data on oral antioxidants, particularly in skin tissue, are needed to provide additional supportive evidence. Nevertheless, this study serves as an important starting point for clarifying the mechanisms underlying the beneficial effects of oral antioxidants on skin.

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