Long-Term Ingestion of High Flavanol Cocoa Provides Photoprotection against UV-Induced Erythema and Improves Skin Condition in Women[1]

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ABSTRACT Dietary antioxidants contribute to endogenous photoprotection and are important for the maintenance of skin health. In the present study, 2 groups of women consumed either a high flavanol (326 mg/d) or low flavanol (27 mg/d) cocoa powder dissolved in 100 mL water for 12 wk. Epicatechin (61 mg/d) and catechin (20 mg/d) were the major flavanol monomers in the high flavanol drink, whereas the low flavanol drink contained 6.6 mg epicatechin and 1.6 mg catechin as the daily dose. Photoprotection and indicators of skin condition were assayed before and during the intervention. Following exposure of selected skin areas to 1.25 × minimal erythemal dose (MED) of radiation from a solar simulator, UV-induced erythema was significantly decreased in the high flavanol group, by 15 and 25%, after 6 and 12 wk of treatment, respectively, whereas no change occurred in the low flavanol group. The ingestion of high flavanol cocoa led to increases in blood flow of cutaneous and subcutaneous tissues, and to increases in skin density and skin hydration. Skin thickness was elevated from 1.11 ± 0.11 mm at wk 0 to 1.24 ± 0.13 mm at wk 12; transepidermal water loss was diminished from 8.7 ± 3.7 to 6.3 ± 2.2 g/(h · m^2) within the same time frame. Neither of these variables was affected in the low flavanol cocoa group. Evaluation of the skin surface showed a significant decrease of skin roughness and scaling in the high flavanol cocoa group compared with those at wk 12. Dietary flavanols from cocoa contribute to endogenous photoprotection, improve dermal blood circulation, and affect cosmetically relevant skin surface and hydration variables. J. Nutr. 136: 1565–1569, 2006.

KEY WORDS: • *epicatechin* • *photoprotection* • *skin* • *human* • *flavonoids* • *blood flow*

Skin is the largest organ of the body, serving as a protective shield against light, heat, injury, and infection. Skin is involved in the regulation of body temperature, water, and lipid stores. Structure, texture, thickness, density, hydration, color, and shielding properties of the skin change with age and vary depending on endogenous and exogenous factors. The nutritional status of the organism affects skin condition (1).

Vitamins and micronutrients have been used in systemic and topical photoprotection. Dietary photoprotection through the administration of carotenoids, tocopherol, and vitamin C in foods or supplements has been successfully used to prevent UV-induced erythema (sunburn) (2). Because most of the annual UV dose is encountered in the absence of topical sun protectants (3), a dietary approach to improve endogenous defense may represent a suitable strategy for preventing the damaging effects of sunlight. Although UV exposure initiates photochemical reactions that lead to damage of light-exposed tissues, it also triggers adaptive mechanisms to escape light-induced stress (4,5). Lipids, proteins, and DNA are cellular targets of photo-oxidation, and damage to these molecules is involved in the pathobiochemistry of erythema formation, premature aging of the skin, development of photodermatosis, and skin cancer. Whereas the photoprotective properties of carotenoids, vitamin E, and vitamin C have largely been attributed to their antioxidant activities (6), these micronutrients may also offer protection by interfering with cellular signaling in UV-dependent responses of the tissue (7).

Flavonoids, a subclass of polyphenols, are secondary plant metabolites found in many commonly consumed fruits and vegetables and, as such, are abundant in a plant-rich human diet (8). Many flavonoids are efficient antioxidants in vitro, and the in vitro antioxidant capacity of a number of fruits and vegetables can be attributed, in part, to flavonoid constituents (9,10). In addition to their putative function as antioxidants, flavonoids can modulate enzyme activity, influence anti-inflammatory pathways, and affect cell division (11). Apart from fruits and vegetables, important sources of flavonoids in human nutrition are cocoa, tea, and red wine (12,13).

Animal studies provide evidence that tea flavanols, when applied orally or topically, ameliorate adverse skin reactions following UV exposure, including skin damage, erythema, and lipid peroxidation (14,15). Topical application of green tea polyphenols to human skin inhibits the UVB-induced erythema response and decreases formation of cyclobutane pyrimidine dimers in skin, both in epidermis and dermis (16). Pretreatment of skin with green tea extracts leads to fewer sunburn cells after

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exposure to solar-simulated radiation with 2 × minimal erythemal dose (MED)[3] and protects epidermal Langerhans cells from UV damage (17). Thus, there is evidence to support the concept that the consumption of dietary flavonoids may confer photoprotection and improve skin quality. The present study was designed to investigate the effects of repetitive intake of a product rich in cocoa flavanols on skin sensitivity toward UV exposure, skin structure, and texture.

MATERIALS AND METHODS

Study design. A total of 24 female volunteers between ages 18 and 65 y, with healthy, normal skin of type II, as described by Fitzpatrick and Pathak (18), were included in the study. Exclusion criteria included pregnancy and breast-feeding, smoking, intake of medication that might influence the outcome of the study, sunbathing, or the use of sunbeds. Participants were advised not to change their dietary habits. No supplements (vitamins, polyphenols, etc.) were allowed during the study. The women ($n = 12$/group) were randomly assigned to either the high flavanol cocoa group (HF) or the low flavanol cocoa group (LF). The study design was double blind.

The HF group ingested a cocoa beverage providing 329 mg of total cocoa flavanols/d for 12 wk. The LF group ingested a nutrient-matched cocoa beverage providing 27 mg of total cocoa flavanols/d for the same period of time. The major flavanol, epicatechin, comprised 19% of total cocoa flavanols in the HF group and 24% in the LF group. The beverage was provided as dry powder and was dissolved in 100 mL of hot water prior to ingestion. The drink was consumed with a meal every morning for the 12-wk duration of the study. Both preparations were provided by Mars Inc. Details on the composition of the cocoa beverages used in the study are given in **Table 1**.

On wk 0, and at the end of wk 6 and 12, the following variables relating to photoprotection and skin health were determined: sensitivity toward UV irradiation, cutaneous blood flow, skin structure and texture, skin hydration, and transepidermal water loss. Compliance was assessed by interview and a counting of the remaining beverage packages. Written informed consent was obtained from each participant, and the study design was approved by the Ethical Committee of the University of Witten-Herdecke, Germany.

Sensitivity toward UV irradiation. The MED was determined for each subject prior to the start of the study. Irradiation to induce erythema (1.25 × MED) was applied to dorsal skin (back and scapular region) using a blue-light solar simulator (Sol 3, Hönle). At each time point (wk 0, 6, and 12) skin color was measured before, and 24-h after, irradiation. Skin color was evaluated by chromametry (Minolta CR 300) using the three-dimensional color system (*L*-, *a*-, and *b*-values). The *L*-value is a parameter for lightness of skin and the *b*-value (blue/yellow axis) indicates pigmentation. The *a*-value (red/green axis) is a measure for erythema and is used to quantify skin responses to UV irradiation. Decreasing *a*-values indicate a photoprotective effect.

Cutaneous blood flow, hemoglobin concentration and oxygen saturation. The O2C-system (Lea Instruments) was used to determine peripheral blood flow and the oxygen saturation of hemoglobin. The measurement of blood flow (arbitrary units) is based on the Doppler effect; the frequency of light is shifted by the moving erythrocyte depending on its velocity. The hemoglobin concentration and oxygen saturation were determined spectroscopically in different skin layers (1 mm and 7–8 mm depths) (19).

Skin structure and texture. High-frequency Ultrasound B-Scan (frequency of 20 MHz-Derma Scan C, version 3) with 2-D configuration (Cortex Technology) was used to analyze tissue structures and obtain information on skin density (pixel) and thickness (mm) (20). Skin surface profiles were evaluated using the SELS (surface evaluation of living skin) method (Visioscan, Courage & Khazaka Electronics) in a 15 × 17 mm area. Four different parameters were used to characterize skin surface: roughness, scaling, smoothness, and wrinkles.

[3] Abbreviations used: HF, high flavanol; LF, low flavanol; MED, minimal erythemal dose; SELS, surface evaluation of living skin.

TABLE 1

Composition of the cocoa powder used to prepare the beverages (18 g/100 mL of water)

Component	HF	LF
	units/serving	
Energy, *kJ*	222	239
Total Fat, *g*	1.0	1.0
Sodium, *mg*	60	140
Total Carbohydrates, *g*	9.0	9.0
Fiber, *g*	4.0	4.0
Sugar, *g*	5.0	5.0
Protein, *g*	5.0	5.0 g
Caffeine, *mg*	10.6	12.3
Theobromine, *mg*	195	190
Total Cocoa flavanols, *mg*	328.5	26.8
Epicatechin (monomer), *mg*	61.1	6.6
Catechin (monomer), *mg*	20.4	1.6
Dimer, *mg*	71.7	7,7
Trimer, *mg*	58.0	4,8
Tetramer, *mg*	50.8	4,0
Pentamer, *mg*	34.2	2,1
Hexamer, *mg*	17.9	0
Heptamer, *mg*	11.5	0
Octamer, *mg*	2.9	0

Skin hydration and transepidermal water loss. Skin hydration (arbitrary units) was determined by corneometry (Corneometer CM 825, Courage & Khazaka Electronics) and transepidermal water loss (TEWL, g/(h · m^2)) was measured using a TEWA-Meter TM 300 (Courage & Khazaka Electronics) (21,22).

Statistics. For all variables, descriptive statistics (means, standard deviation, minimum, lower quartile, median, upper quartile, and maximum) were calculated at 3 time points (wk 0, wk 6, and wk 12). For all variables, before and after differences for each combination of two time points were calculated. Within the two treatment groups, each combination of two time points was compared using Wilcoxon's Signed Rank test. The before and after differences of the two treatment groups were compared using Wilcoxon's Rank Sum test. Differences were considered significant at $P < 0.05$.

RESULTS

The cocoa beverages used in the high and low flavanol group were comparable with respect to their constituents except for the flavanol content (Table 1). The HF cocoa group received a daily dose of 329 mg total flavanols, whereas the LF cocoa group received only 26.8 mg. The daily doses of epicatechin and catechin in the HF group were 61.1 mg and 20.4 mg, and 6.6 mg and 1.6 mg in the LF group, respectively. Making up the difference for total flavanols, the daily dose of procyanidins (oligomers) was 247 mg in the HF group and 18.6 mg in the LF group.

Photoprotection. Protection by dietary cocoa flavanols against UV-induced skin responses (erythema) was measured as a decrease in reddening following exposure of selected skin areas toward 1.25 × MED of solar-simulated radiation. Reddening after UV exposure was determined by chromametry. Chromametry *a*-values taken 24-h after irradiation, and the difference between chromametry *a*-values taken before and after irradiation (Δ-*a* value), were used as a measure for UV response of the skin (**Table 2**). In the high flavanol cocoa group, the Δ-*a* value is lower after 6 wk ($P = 0.001$) and 12 wk ($P = 0.012$) of treatment than at the beginning of the study. The *a*-values taken 24-h after irradiation were ~15 and 25%

TABLE 2

Sensitivity of skin to irradiation with UV light (1.25× MED) in women at wk 0 and after 6 and 12 wk of consuming HF or LF cocoa beverages[1]

	Time, *wk*		
	0	6	12
	a-value		
HF			
Before irradiation	7.7 ± 2.2	8.2 ± 2.0	7.9 ± 1.9
24 h after irradiation	12.5 ± 1.8	10.5 ± 2.1[2,3]	9.4 ± 1.8[2,3]
Δ-*a* value	4.8 ± 1.4	2.3 ± 1.8[2,3]	1.5 ± 0.7[2,3]
LF			
Before irradiation	7.4 ± 1.9	8.0 ± 1.6	7.4 ± 2.2
24 h after irradiation	11.1 ± 2.7	11.2 ± 2.8	11.9 ± 2.8
Δ-*a* value	3.7 ± 1.3	3.2 ± 1.4	4.5 ± 1.6

[1] Values are means ± SD, $n = 12$.
[2] Different from wk 0, $P < 0.05$.
[3] Change compared with wk 0 is significantly different from the LF group, $P < 0.05$.

lower at wk 6 and 12, respectively, than at the beginning of the study ($P < 0.05$). The 24-h *a*-values and Δ-*a* values did not change in the low flavanol cocoa group during the 12-wk treatment. Thus, consumption of a flavanol-rich cocoa beverage provides photoprotection, whereas a similar cocoa beverage, low in flavanols, does not.

Cutaneous blood flow. Following supplementation with the HF cocoa beverage for 6 and 12 wk, an increase in blood flow occurred in cutaneous (1 mm) and subcutaneous (7–8 mm) tissue ($P < 0.05$, **Table 3**). Blood flow did not change in the LF group. Hemoglobin concentrations did not change in either group (data not shown).

Skin structure and texture. In women supplemented with HF cocoa beverage, moderate but significant increases in density and thickness of the skin occurred at 6 and 12 wk (**Table 4**). These variables did not change in the LF group. Typical examples of ultrasound B-scans before and 12-wk after supplementation are shown (**Fig. 1**). Using the SELS method, a significant decrease in skin roughness and scaling was measured at 12 wk in the HF group, whereas no change was found in the LF group (Table 4). The SELS parameters for smoothness and wrinkles did not change in either of the intervention groups.

TABLE 3

Relative peripheral blood flow in skin of women at wk 0 and after 6 and 12 wk of consuming HF or LF cocoa beverages[1]

	Time, *wk*		
Skin depth, *mm*	0	6	12
	arbitrary units		
HF			
1	16 ± 7	24 ± 12[2,3]	32 ± 16[2,3]
7–8	133 ± 57	155 ± 61[2]	183 ± 66[2,3]
LF			
1	17 ± 9	17 ± 6	16 ± 6
7–8	144 ± 45	134 ± 50	131 ± 47

[1] Values are means ± SD, $n = 12$.
[2] Different from wk 0, $P < 0.05$.
[3] Change compared with wk 0 is significantly different from LF group, $P < 0.05$.

TABLE 4

Variables related to skin structure and texture determined by utltrasound B-scan surface evaluation of the skin and corneometry of women at wk 0 and after 6 and 12 wk of consuming HF or LF cocoa beverages[1]

	Time, *wk*		
	0	6	12
	arbitrary units		
HF			
Density, *pixel*	10.2 ± 1.7	11.3 ± 2.1[2,3]	11.9 ± 1.6[2,3]
Thickness, *mm*	1.11 ± 0.11	1.20 ± 0.14[2,3]	1.24 ± 0.13[2,3]
Roughness, *AU*	0.27 ± 0.20	0.20 ± 0.17	0.19 ± 0.18[2]
Scaling, *AU*	0.14 ± 0.09	0.10 ± 0.07	0.08 ± 0.06[2]
Smoothness, *AU*	20.3 ± 1.9	20.9 ± 1.9	21.2 ± 2.5
Wrinkles, *AU*	42.2 ± 5.1	41.8 ± 4.1	41.8 ± 4.1
Hydration, *AU*	39 ± 4	40 ± 6	44 ± 8[2,3]
Transepidermal water loss, *g/(h · m²)*	8.7 ± 3.7	7.8 ± 3.5	6.3 ± 2.2[2,3]
LF			
Density, *pixel*	12.5 ± 1.2	12.3 ± 1.4	12.4 ± 1.2
Thickness, *mm*	1.05 ± 0.10	1.05 ± 0.10	1.04 ± 0.11
Roughness, *AU*	0.13 ± 0.20	0.17 ± 0.17	0.15 ± 0.13
Scaling, *AU*	0.18 ± 0.22	0.11 ± 0.08	0.13 ± 0.11
Smoothness, *AU*	19.6 ± 3.1	20.7 ± 2.1	20.5 ± 1.9
Wrinkles, *AU*	44.4 ± 5.4	44.0 ± 5.1	43.7 ± 4.4
Hydration, *AU*	38 ± 5	36 ± 4	36 ± 6
Transepidermal water loss, *g/(h · m²)*	7.2 ± 4.2	7.4 ± 3.2	6.9 ± 2.0

[1] Values are means ± SD, $n = 12$.
[2] Different from wk 0, $P < 0.05$.
[3] Change compared with wk 0 is significantly different from LF group, $P < 0.05$.

Examples of SELS profiles before and after supplementation are shown (**Fig. 2**). Skin hydration was significantly increased after 12 wk in women that consumed the HF cocoa beverage, whereas it was not affected in the LF group (Table 4). Transepidermal water loss was significantly decreased at wk 12 compared with baseline in the HF group; no change between baseline and wk 12 occurred in the LF group.

DISCUSSION

Photoprotection. Based on human intervention studies with several antioxidant micronutrients, including carotenoids, tocopherol, and ascorbate, the concept that foods and supplements may offer a dietary approach to photoprotection has gained momentum (2). Animal studies, and results from topical application studies in humans, provide evidence that flavonoids, especially members of the flavanol family, can also offer effective photoprotection (23). We showed here for the first time, to our knowledge, that dietary intervention with a cocoa beverage rich in flavanols decreased the sensitivity of human skin toward UV light, which was determined by the degree of erythema (reddening) following irradiation with a solar light simulator. Compared with baseline, the skin response was decreased by 15% after 6 wk of intervention, and the decrease was more pronounced, 25%, after 12 wk. UV sensitivity did not change in the women that consumed the cocoa beverage low in flavanols. Compared with the low flavanol product, the high flavanol cocoa powder contained ~10× the amount of epicatechin and catechin, as well as total flavanols (Table 1).

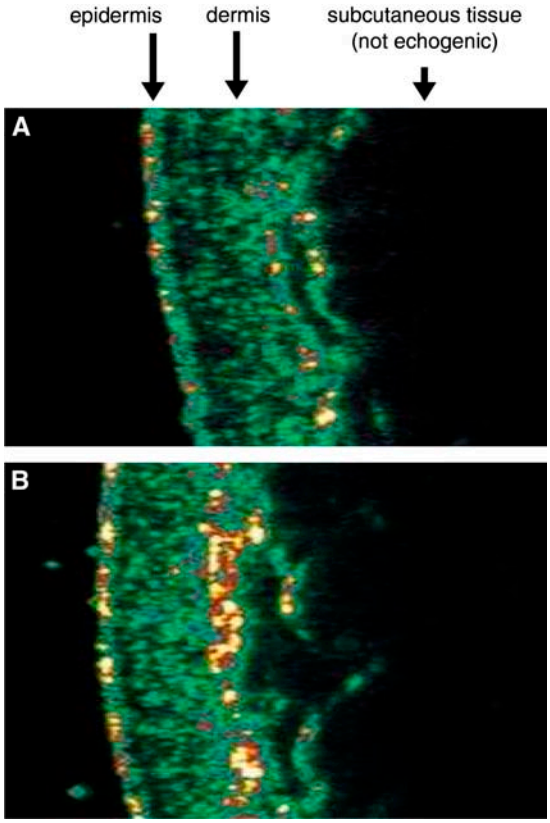

FIGURE 1 Examples of high-frequency ultrasound B-scans showing skin density of women at wk 0 (*A*) and after 12 wk of consuming high flavanol cocoa beverages (*B*) (see Table 4).

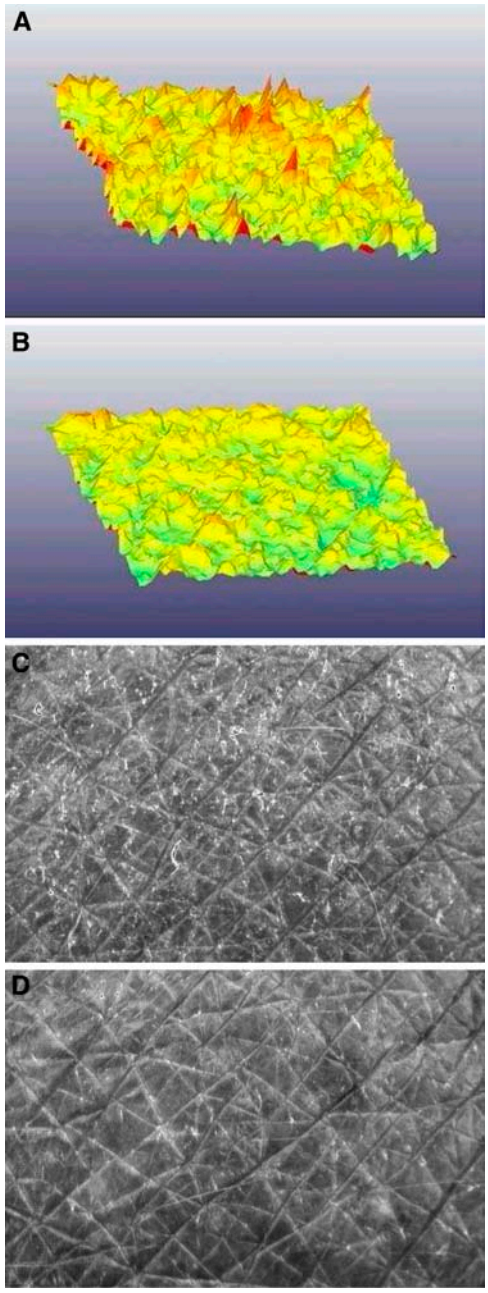

FIGURE 2 Examples of skin surface profiles of women evaluated with the surface evaluation of living skin method at wk 0 (*A*) and after 12 wk of consuming high flavanol cocoa beverages (*B*). The corresponding top view of the skin is shown in the lower photographs (*C*, *D*).

These amounts are similar to those found in 100 g of dark chocolate (12). The photoprotective effect of the high flavanol cocoa product is comparable to that reported for carotenoid supplements (24). After consuming 24 mg of β-carotene/d or 24 mg of mixed carotenoids, the chromametry a-values at 12 wk were 20–25% lower than baseline. Similar results were obtained after ingesting lycopene-rich tomato products (25).

Carotenoids and flavanols are efficient antioxidants and contribute to photoprotection in plants (26,27). They can scavenge primary or secondary reaction products of photo-oxidation, such as singlet molecular oxygen or peroxyl radicals. Most polyphenols absorb UV light, and this shielding process may also contribute to photoprotection. At a micromolar level, flavanols and procyanidins protect DNA from oxidation following UV-C irradiation in vitro (28). Dietary antioxidants exhibit other biological properties as well; for example, they affect intra- and intercellular signaling. UV-induced erythema is an inflammatory event associated with complex biochemical processes of this tissue response. Certain flavonoids are potent inhibitors of the production of prostaglandins and interfere with key enzymes involved in prostaglandin biosynthesis (29).

Cutaneous blood flow. Microcirculation in skin is complex, and it is organized into a superficial and deep plexus. Microcirculation is important for thermoregulation, nutrient and oxygen supply, and it affects skin condition and appearance (30). Several drugs, applied topically or systemically, modulate skin blood flow; cutaneous blood vessels respond to vasoactive agents, like acetylcholine or sodium nitroprusside (31,32). In the present study, we found an increase in cutaneous and subcutaneous blood flow in women supplemented for 12 wk with a cocoa beverage rich in flavanols. At wk 12, blood flow increased ~100% at 1 mm depth and ~40% at 7–8 mm depth compared with baseline. The effect was less pronounced but was significant after 6 wk of intervention. No change occurred in women supplemented with the low flavanol cocoa beverage. Vasodilation by flavanol-rich cocoa has been shown in healthy humans by examining flow-mediated dilation of the brachial artery (33) and by finger pulse wave amplitude analysis (34). The pool of bioactive NO was increased after consuming a flavanol-rich cocoa drink. The increase in the circulating bioactive NO pool may contribute to the beneficial vascular health effects of flavanol-rich food. These changes were correlated with increasing levels of flavanol metabolites, and they were mimicked by epicatechin (35).

Skin structure and texture. Maintaining skin integrity is vital to overall health and requires an optimal supply of

nutrients. We show here that consuming cocoa flavanols improves skin texture, mainly, its density, thickness, roughness, and scaling (Table 4). Skin hydration is also improved and transepidermal water loss is decreased. The underlying mechanisms are not known; however, the flavanol-mediated increase in cutaneous blood flow likely contributes to an improvement in skin appearance.

In summary, dietary constituents can protect skin as well as improve overall skin quality. This study demonstrates that the regular consumption of a beverage rich in flavanols can confer substantial photoprotection as well as help maintain skin health by improving skin structure and function. The photoprotection offered by cocoa flavanols is within the range of that reported for dietary carotenoids, such as β-carotene or lycopene.

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