Original Contribution

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Reduction of facial wrinkles depth by sleeping on copper oxide-containing pillowcases: a double blind, placebo controlled, parallel, randomized clinical study

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Summary

Background Copper up-regulates the secretion of extracellular skin proteins and stabilizes the extracellular matrix once formed. As copper can be absorbed through intact skin, we reasoned that sleeping on pillowcases containing copper-impregnated fibers would reduce skin wrinkles.
Objective Demonstrate that sleeping on pillowcases containing copper-impregnated fibers reduce facial skin wrinkles.
Patients/Methods An 8-week, double blind, parallel, randomized study was carried out, in which healthy volunteers, aged 30–60, used either copper oxide-containing pillowcases (1% weight/weight) (test group, $n = 30$) or control pillowcases without copper (control group, $n = 31$). Skin conditions of the subjects were evaluated by visual grading by two expert graders and by 3D Image Analysis GFM PRIMOS® at baseline (before treatment) and following 4 and 8 weeks of sleeping on the pillowcases.
Results The use of the copper oxide-containing pillowcase resulted in significant decrease of crow's feet after 4 ($P = 0.01$) and 8 ($P = 0.002$) weeks, but none was observed in the control group, as determined by the expert graders. On the basis of the 3D measurements, three roughness (R) parameters were improved after 4 and 8 weeks ($P < 0.02$) and the Rmax parameter at 8 weeks ($P = 0.016$) in the test group, but there were no changes in the R-parameters during the course of the study in the control group. The average reduction per month in the R-parameters was approximately 9%. No adverse reactions were observed or reported during the 8 weeks study.
Conclusions Sleeping on copper oxide-containing pillowcases results in reduction of wrinkles depth and overall improvement of skin appearance.

Keywords: copper oxide, wrinkles, pillowcases, textile, clinical study

Introduction

Copper is an essential trace element involved in numerous human physiological and metabolic processes,[1] including in synthesis and stabilization of extracellular matrix (ECM) skin proteins critical for skin formation[2,3] and wound repair.[4,5] The recommended daily allowance of copper is about 1 mg and copper salts, such as copper oxide, can be found in food additives.

Copper is safe for human use. For example, copper intrauterine devices for the prevention of inception are widespread and used safely for very prolonged periods of time by millions of women worldwide. In anthroposophical medicine, copper is used orally, as subcutaneous

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injections, and as ointments as a means for stimulating the body to heal itself. Copper ointments, with copper concentrations that can reach up to 20%, are used, for example, in the treatment of cramps, disturbances of renal function, peripheral, venous hypostatic circulatory disturbances, rheumatic disease, and swelling associated with trauma.[6] The risk of adverse reactions owing to dermal contact with copper is extremely low.[7] Applications of ointment preparations containing copper in concentrations up to 20% did not cause any adverse reactions or toxicity.[8]

Copper oxide particles can be permanently introduced into textile products endowing them with a broad spectrum of anti-microbial and anti-fungal properties.[9] For example, it has been demonstrated in a clinical trial with 56 patients that by wearing copper oxide-containing socks, the clinical manifestations of acute or chronic fungal infections are significantly improved or resolved because of the potent antifungal efficacy of the socks.[10] These products do not cause skin sensitization or irritation.[9–11]

On the basis of the known role that copper plays in skin regeneration and on the capacity of facial skin to absorb copper,[8,12] we have hypothesized that sleeping on pillowcases containing copper would have a significant positive cosmetic effect on the individuals using them. Our previous study, based on the visual grading by two experts of photographs taken from 60 individuals at baseline or after using placebo or pillowcases containing copper oxide, strongly supported our hypothesis.[11] However, this study had its limitations, as it was not based on quantitative measurements. In the present study, using an ISO 9001:2000 certified human skin safety and efficacy skin research center (Dermapro), and following Good Clinical Practice Regulations, this finding was not only confirmed by the actual physical examination of the panelists of expert graders, but the reduction of crow's feet fine lines that occurred following the use of copper oxide-containing pillowcases was quantified by specialized three-dimensional *in vivo* measurement system (PRIMOS) developed to measure wrinkles size.[13]

Methods

Participants

Sixty-two subjects were recruited on the basis of the inclusion and exclusion criteria detailed in Table 1. Each volunteer was informed of the purpose and the protocol of the study, the study timetable, the possible benefits, the constraints linked to the study, and the possible risks. After making a completely free decision, each participant willing to participate in the study signed an informed consent.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Non-inclusion criteria
Healthy women, between 30 and 60 years of age.	Pregnancy or nursing condition.
Individuals with a fine line/wrinkle score of ''4'' (noticeable) or greater in a severity scale of 0 (minimum) to 9 (maximum) at the crow's feet area around the eyes, as evaluated by expert graders.	Medication or past medical history that may affect skin response.
	Any active skin disease that may interfere with the aim of the study.
	Treatment of immuno-suppression within 3 months.
Signed informed consent after the purpose and the protocol of the study were explained.	Participation in a previous study without an appropriate 3 months rest period in the interim.
Volunteers should be cooperative and available during the study period.	Chronic sickness (diabetes, asthma, high blood-pressure).
	Serious renal disorder or hepatic dysfunction.
	Individual has damaged skin in or around the test site, which includes sunburn, uneven skin tones, tattoos, scars or other disfiguration of the test site.

Test items

Two test items (TI) were included in the study: a polyester pillowcase containing 1% (weight/weight) copper oxide particles impregnated in the yarn (TI1; Fig. 1) and a control polyester pillowcase not containing copper oxide (TI2). Each volunteer was given, based on block randomization, either two TI1 or two TI2, without the volunteer or the researchers knowing if TI1 or TI2 were the control or test item. The participants were required to use only the pillowcases they received (either TI1 or TI2) solely every night during the whole study as they would normally use their own pillowcase during their sleep. In addition, all study participants were given a cleanser (Neutrogena sensitive skin solutions foam cleanser) and oil-free moisturizer (Neutrogena skin clearing toner) and requested only to use only these products and not any other cleansing or moisturizing products during the entire study period. The participants were instructed to wash and dry the pillowcases as they would normally do with their own laundry, but without using any softener.

Evaluation of crow's feet wrinkles

Before and after 4 and 8 weeks of sleeping on the pillowcases, the facial skin and in particular the crow's feet of each participant was evaluated both by visual

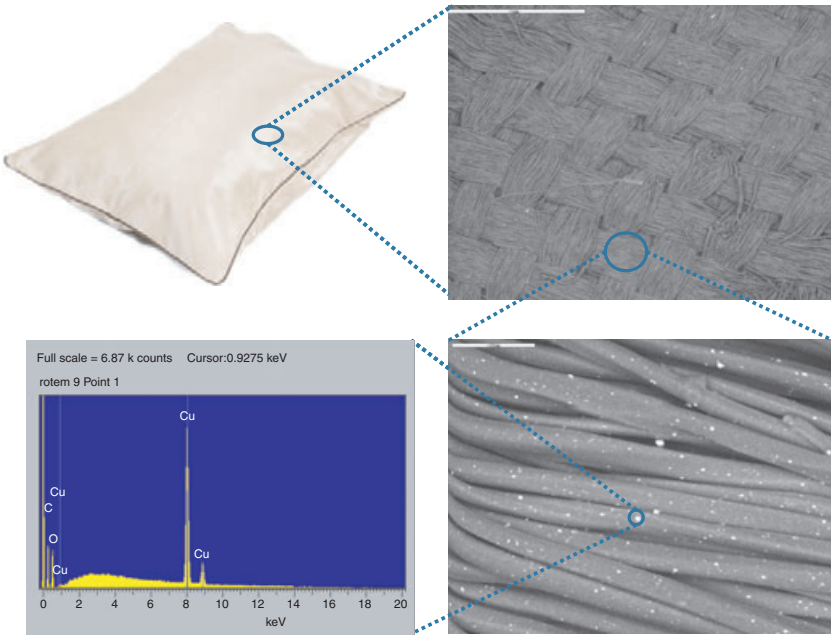

Figure 1 Two scanning electronic microscope images (taken with a Jeol JMS 840 scanning electron microscope) of the test pillowcase are shown. The white dots are the copper oxide particles embedded in the polyester fibers. The chart is an X-ray photoelectron spectra analysis (performed with a Shimadzu XRD 6000, TN-5500 X-ray analysis system) of the encircled white dot, showing a peak at 8 keV corresponding to copper.

grading by expert graders and by a 3D Imaging system, as detailed below. In the case of the one-side (right or left) sleepers (as indicated by the participants themselves), the scoring values of the relevant side were only tabulated, and as for the both-side sleepers, both sides were tabulated and the average values were given per participant. The evaluations were performed under controlled and identical environmental conditions for all participants—the ambient temperature was maintained at 22 ± 2 °C and the relative humidity in the range of 50 ± 5%. Before examination, all participants did not wear makeup and were asked not to wash their faces; then they were allowed to relax on a comfortable diagnostic bed.

Visual grading

The visual grading of the wrinkles around the eyes was evaluated by two trained researchers according to a 10 detailed criteria scale, being 0 as having ''no wrinkles'' and 9 as the most severe wrinkles score (very deep wrinkles with redundant folds).

3D image analysis

Pictures of the crow's feet were captured with a Facial Stage® DM-3 5 megapixel camera (Moritex, Azamino-minami YokohamaAoba Ward, Kanagawa Prefecture, Japan) and four crow's feet roughness parameters (Ra, Rmax, Rz and Rp; Table 2) were evaluated by using a 3D Image Analysis GFM Phaseshift Rapid *in vivo* Measurement of Skin (PRIMOS)® system (GFMesstechnik GmbH, Warthestraβe, Teltow, Germany). This device, which allows fast, contact-free, and direct measurement of the skin surface topography *in vivo* at high resolution, is less artifact prone and more accurate method than the

Table 2 Definition of roughness parameters measured

Parameter	Name	Definition
Ra	Average roughness	Arithmetic average value of profile peaks within the total measuring length of the wrinkle
Rmax	Maximum roughness depth	Maximum of all peak-to-valley values measured over the assessment length of the wrinkle
Rz	Mean roughness depth	Average maximum height of the profile of 5 consecutive measures of a wrinkle. Rz represents the vertical distance between the highest peak and the deepest valley within the sampling length of a wrinkle
Rp	Maximum base roughness depth	Largest single base roughness depth within the total evaluation length of a wrinkle

commonly used silicon replica technique.[14,15] The measurement is based on the projection of digital light stripe patterns onto the skin surface followed by recording the projected stripes by a camera at a different angle. Employing complex mathematical algorithms, the difference between the projected and recorded light stripe patterns leads to the 3-dimensional skin surface topography, which allows measuring of the depth of fine skin lines and wrinkles. In addition, the very short measuring time further guarantees that the captured data is not significantly influenced by involuntary movements of the subject. The optical system was fixed on a 3D stereotactic face device allowing positioning of the face in a reproducible manner.

Adverse reactions

The safety of the product was assessed both by clinical observation by a dermatologist and by feedback obtained from the trial participants. The objective signs examined were erythema, edema, scaling, and papule. The subjective signs included were as follows: itching, prickling, burning, stinging, stiffness, tightness, burning of eyes, and weeping. The frequency, duration, and intensity of each given sign and a possible or probable relationship with the test product were investigated.

Statistical analysis

Paired *t*-test and ANCOVA analyses were conducted using SPSS® (IBM, New Orchard Road, Armonk, NY, USA) 11.5 software program. A statistically significant difference was set at $P < 0.05$. The increase or decrease of a given parameter was calculated according to the following equation:

$$(\text{Initial score at baseline} - \text{score after use (4 or 8weeks)}/\text{Initial score at baseline}) \times 100$$

Results

Skin characteristics of subjects

Of 62 subjects recruited, 61 finished the trial. One subject dropped out for personal reasons. The age of the 61 subjects that finished the trial varied between 35 and 56 years (average 46.75 ± 4.15 years of age). The facial skin characteristics and sleeping habits of all participants are summarized in Table 3. The subjects were divided into two groups following a computerized randomization. There were no statistical differences between both groups in terms of the facial skin characteristics at baseline as determined both by visual grading and 3D imaging. In the control and test groups 8 and 9 participants respectively reported that they sleep either on their right or left side only. The rest of the participants reported that they sleep on both sides of their face. In the control and test group 24 and 26 participants respectively reported that they sleep between 5 and 8 h per night, 2 in each group reported that they sleep <5 h per night, and 3 and 4 participants reported that they sleep more than 8 h per night.

Table 3 Skin characteristics and sleeping habits of participants

Item	Classification	Frequency (*n*)	Percentage (%)
Skin type	Dry	24	39.34
	Normal	18	29.51
	Oily	4	6.56
	Dry and oily	15	24.59
	Problematic	0	0
Hydration	Sufficient	1	1.64
	Normal	33	54.1
	Deficient	27	44.26
Sebum	Glossy	10	16.39
	Normal	35	57.38
	Deficient	16	26.23
Surface	Smooth	21	34.43
	Normal	31	50.82
	Rough	9	14.75
Thickness	Thin	22	36.07
	Normal	33	54.1
	Thick	6	9.84
Time of UV exposure per day	<1 h	19	31.15
	1–3 h	37	60.66
	Above 3 h	5	8.2
Time of sleeping per night	<5 h	4	6.56
	5–8 h	50	81.97
	Above 8 h	7	11.48
Sleeping habits	Front side	0	0
	Left side	9	14.75
	Right side	9	14.75
	Both sides	43	70.49
Irritability	Yes	12	19.67
	No	49	80.33
Stinging	Yes	4	6.56
	No	57	93.44

Evaluation of crow's feet reduction by visual grading

Compared to baseline (week 0), there was a statistically significant reduction in the crow's feet after 4 weeks ($P = 0.01$) and 8 weeks ($P = 0.002$) in those using the pillowcase containing copper oxide, whereas there was no changes in the crow's feet in those using the control pillowcases (Table 4). The difference between both groups reached statistical significance at 8 weeks ($P = 0.04$), while at 4 weeks the *P*-value was 0.053 (Table 5).

Table 4 Comparison of the visual grading before and after using the pillowcases

Group	*N*	Week	Mean†	SD	SEM	*P*-value	Decrement (%)
TI1	30	0	5.97	1.21	0.22	–	–
		4	5.89	1.2	0.22	0.01*	1.26▼
		8	5.73	1.17	0.21	0.002*	3.91▼
TI2	31	0	5.77	1.07	0.19	–	–
		4	5.78	1.06	0.19	0.787	0.14△
		8	5.82	1.13	0.20	0.732	0.87△

†Decrement (▼) of the mean value represents improvement of skin wrinkles.
*Significantly different at $P < 0.05$ compared with before using the pillowcase.

Table 5 Comparison of visual scores between the two groups (TI1 *vs.* TI2) at 4 and 8 weeks

Week	Type III sum of squares	df	Mean square	*F*	*P*-value
4 weeks	0.096	1	0.096	3.899	0.053
8 weeks	1.136	1	1.136	9.19	0.004*

*Statistically different at $P < 0.05$ compared with control group.

Evaluation of crow's feet reduction by 3D *in vivo* optical skin imaging

Compared to baseline (week 0), there was a statistically significant reduction of approximately percentage in the Ra, Rp, and Rz parameters ($P < 0.01$; Table 6) already after 4 weeks in the group using TI1, as determined by 3D image analysis (Fig. 2). In the same group, there was a trend of reduction in the Rmax parameter after 4 weeks ($P = 0.094$; Table 6) reaching statistical significance at 8 weeks ($P = 0.028$; Table 6). In contrast, there were no changes in the R-parameters in the group sleeping on the control TI2 both at 4 and 8 weeks (Table 6). Accordingly, there was a statistically significant difference between both groups in the Ra, Rp, and Rz parameters at 4 and 8 weeks and in the Rmax parameter at 8 weeks (Table 7).

Adverse reactions

On the basis of both the participants reports as well as by clinical observation by the expert graders, not even one adverse reaction of itching, prickling, burning, sting, stiffness, tightness, burning of the eyes, weeping, erythema, edema, scaling, papule, or any other reaction

Table 6 Comparison of the R-parameters before and after treatment using 3D *In vivo* optical skin imaging

Parameter	Group	*N*	Week	Mean†	SD	SEM	Reduction (%)	*P*-value
Ra	TI1	30	0	23.49	5.85	1.07	–	–
			4	21.33	4.47	0.82	9.19▼	<0.001*
			8	21.51	4.85	0.89	8.42▼	0.001*
	TI2	31	0	22.71	4.70	0.84	–	–
			4	22.38	4.74	0.85	1.46▼	0.493
			8	22.80	6.09	1.09	0.37△	0.883
Rmax	TI1	30	0	192.24	66.49	12.14	–	–
			4	179.68	67.03	12.24	6.53▼	0.094
			8	176.33	56.31	10.28	8.27▼	0.028*
	TI2	31	0	176.51	46.26	8.31	–	
			4	181.64	44.18	7.94	2.90△	0.323
			8	186.08	53.58	9.62	5.42△	0.132
Rp	TI1	30	0	77.22	19.15	3.50	–	
			4	70.82	17.65	3.22	8.29▼	0.001*
			8	70.59	16.59	3.03	8.59▼	0.001*
	TI2	31	0	74.25	13.18	2.37	–	–
			4	77.23	14.83	2.66	4.01△	0.082
			8	79.24	19.07	3.43	6.72△	0.076
Rz	TI1	30	0	130.33	35.57	6.86	–	–
			4	117.51	29.96	5.47	9.84▼	0.002*
			8	117.98	30.09	5.49	9.47▼	0.006*
	TI2	31	0	123.06	27.50	4.94	–	–
			4	122.34	26.90	4.83	0.59▼	0.798
			8	124.84	31.13	5.59	1.45△	0.606

†Decrement (▼) of the mean value represents improvement of skin wrinkles.
*Significantly different at $P < 0.05$ compared with before using the pillowcase.

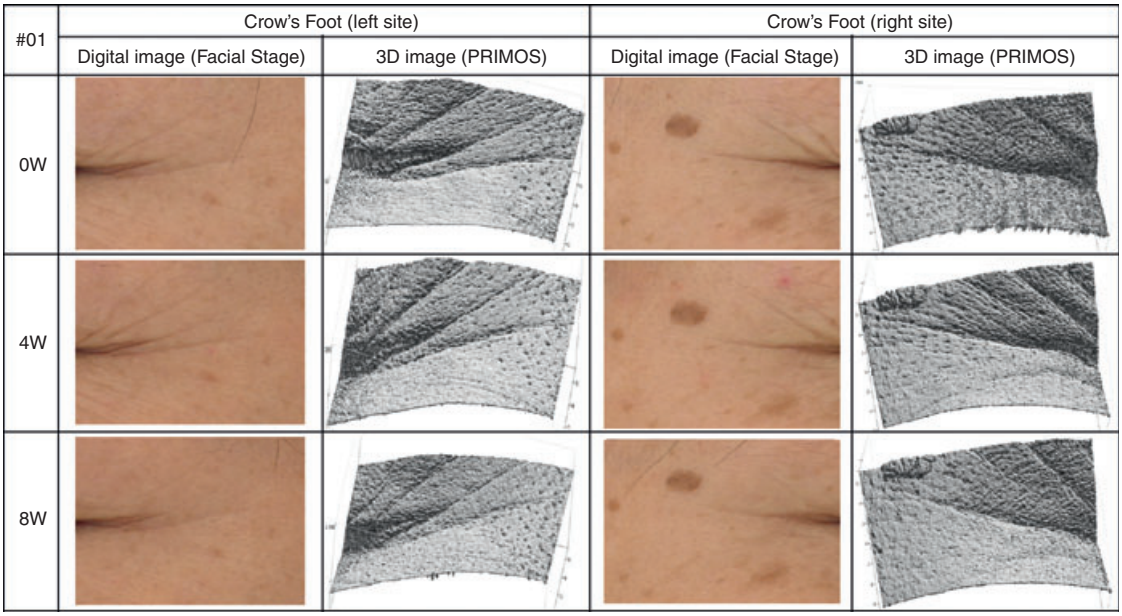

Figure 2 Representative roughness pictures taken by the 3D *in vivo* imaging system of subject number 1 of test group.

Table 7 Comparison of R-parameters of between-groups visual scores between the two groups (TI1 *vs.* TI2) at 4 and 8 weeks

Parameter	Week	Type III sum of squares	df	Mean square	F	*P*-value
Ra	4	40.153	1	40.153	6.753	0.012*
	8	58.559	1	58.559	6.409	0.014*
Rmax	4	3222.994	1	3222.994	2.971	0.09
	8	7073.65	1	7073.65	6.212	0.016*
Rp	4	1183.851	1	1183.851	13.891	<0.001*
	8	1821.491	1	1821.491	11.532	0.001*
Rz	4	1525.992	1	1525.992	6.007	0.017*
	8	2230.235	1	2230.235	6.056	0.017*

*Statistically different at $P < 0.05$ compared with control group.

was noted in all 61 participants both after 4 and 8 weeks of using the control (TI2) or test (TI1) products.

Discussion

As indicated in our previous study,[11] and as clearly demonstrated in this study by two expert graders and most importantly by state-of-the-art quantitative equipment, sleeping on copper oxide-containing pillowcases during 1 month results in reduction in the depth of facial wrinkles. The mean reduction of the depth of the wrinkles was approximately 9%, as determined by the 3D imagining quantification.

We postulate that a possible physiological explanation to the reduction in wrinkles depth is that copper ions liberated into the moisture found between the face and the pillowcase are absorbed through the skin. Once absorbed they induce fibroblast proliferation, and stabilization and stimulation of formation of ECM proteins, such as collagen, fibronectin, and integrin. The above hypothesis is in accordance with the following observations: (i) absorption of copper or copper oxide applied on the facial skin has been demonstrated[8,12]; (ii) copper stimulates dermal fibroblasts proliferation[16]; (iii) dermal fibroblasts are primary cells responsible for ECM maintenance; (iv) copper oxide stimulates *in vitro* the formation of several collagens and elastin by dermal fibroblast (unpublished data); (v) collagen, a component of the ECM, provides, together with other proteins (e.g., fibronectin), structure and strength to skin, whereas elastin provides firmness and resiliency/elasticity characteristics; (vi) several proteins, such as lysyl oxidase, needed for efficient ECM protein cross-linking, including of collagen, elastin and fibronectin, require copper as a cofactor[17]; (vii) stabilization of fibronectin mats[18] and collagen crosslinking is increased by copper ions[19]; (viii) human peptide Gly-(L-His)-(L-Lys) or GHK, when it interacts with copper ions, increases protein synthesis of collagen and elastin[2]; and (ix) Menkes patients (patients with incapacity to metabolize copper) have reduced collagen formation and lysyl oxidase activity.[20] In addition, (i) copper stimulates expression of matrixmetalloproteinase-1

in dermal fibroblasts[16]—the ECM proteins can be degraded or remodeled by the matrixmetalloproteinases; (ii) copper is a strong antioxidant. It works by attaching itself to the enzyme superoxide dismutase present in the skin, important in protection against free radicals[21]; (iii) copper modulates integrins expression by keratinocytes[22]; and (iv) topical copper sulfate treatment accelerates epithelial tissue growth.[23] Accordingly, several commercial facial creams contain copper as their active ingredient (e.g., Neutrogena Visibly Firm® Face Lotion SPF 20, Chetle AveWhittier, CA, USA).

As demonstrated in this study and in the previous study,[11] the use of copper oxide-containing pillowcases did not cause any adverse reactions, such as skin irritation, itching, and burning. This is in accordance with the very low risk of adverse reactions because of dermal copper contact[7] and with the not even one adverse reaction caused by textile products containing copper oxide that were previously tested.[9–11,24,25]

Other possible concerns may be the inhalation of copper oxide particles. However, in an experiment simulating human inhalation conditions for 5 h and using good laboratory practices (GLP) conducted by Nelson Laboratories (Salt Lake City, UT, USA), the amount of copper particles released from copper oxide impregnated fabrics containing two times the amount of copper oxide found in the copper oxide-containing pillowcases, was negligible and well below the USA Occupational Safety and Health Administration (OSHA) permissible exposure limits (PEL) for copper.[24] This experiment indicates that pillowcases containing 1% copper oxide (w/w) do not pose any risk because of possible inhalation of copper particles.

In addition, although copper is an essential element[1] and although copper oxide is found in multivitamin pills and dietary supplements, an experiment using GLP was conducted in an independent laboratory (Nelson Laboratories) in which the amount of copper, eluting into simulated saliva from a fabric impregnated with two times the amount of copper found in the tested copper-containing pillowcases, was determined. This experiment simulates an extreme unlikely scenario whereby secreted saliva coming into contact with the pillowcases during sleep may be ingested. The amount of copper that eluted into the saliva was below the minimal risk level (MRL) for copper oral exposure,[24] determined to be 0.01 mg/kg/day by the Agency for Toxic Substances and Disease Registry of the U.S. Department of Health and Human Services, indicating that even a fabric with two times as much copper as that used in the study would not pose a risk because of possible consumption of the copper that may elute into saliva.

In conclusion, this study confirms our hypothesis that sleeping on fabrics that liberate copper ions reduces the depth of wrinkles. This is a safe and easy way to help improve the well-being of the skin and its appearance.

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