



Improvement of facial skin characteristics using copper oxide containing pillowcases: a double-blind, placebo-controlled, parallel, randomized study

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Synopsis

Copper plays a key role in several processes of skin formation and regeneration. Copper has been shown to be absorbed through intact skin. We hypothesized that sleeping on fabrics containing copper-impregnated fibres would have a positive cosmetic effect on the skin. The aim of this study was to confirm our hypothesis. A 4-week, double blind, parallel, randomized study was carried out in which 57 volunteers aged 40–60 years used either copper oxide containing pillowcases (0.4% weight/weight) or control pillowcases not containing copper. Photographs were taken by a professional photographer of each participant at the beginning of the study and at 2 and 4 weeks after the commencement of the study. Two expert graders (a dermatologist and a cosmetologist) evaluated the pictures for the effect on several cosmetic facial skin characteristics. The copper-containing pillowcases had a positive effect for the following facial characteristics: reduction of wrinkles ($P < 0.001$) and crow's feet/fine lines ($P < 0.001$) and improvement of general appearance ($P < 0.001$) at both 2 and 4 weeks. The differences were statistically significant (Wilcoxon scores and chi-squared tests). Consistent sleeping for 4 weeks on copper oxide containing pillowcases caused a significant reduction in the appearance of facial wrinkles and crow's feet/fine lines

and significant improvement in the appearance of facial skin. In most trial participants, this effect was already noticeable within 2 weeks of using the copper oxide containing pillowcases.

Résumé

Le cuivre joue un rôle significatif dans quelques processus de la formation et la régénération de la peau. Le cuivre était montré d'être absorbé à travers la peau intacte. Nous avons supposé que le sommeil sur les tissus contenant les fibres imprégnées par le cuivre auraient un effet cosmétique positif sur la peau. Le but de cette étude était de confirmer notre hypothèse. Une étude de 4 semaines double-aveugle, parallèle, *et aléatoire* était exécutée, dans laquelle 55 volontaires, âgés 40–60 ans, avaient utilisé soit les taies d'oreiller contenant l'oxide de cuivre (0.4% poids/poids) soit les taies d'oreiller controles qui ne contenaient pas du cuivre. Chaque participant était pris en photo par un photographe professionnel au debut de l'étude et au point de 2 et 4 semaines après le debut de l'étude. Deux correcteurs experts (un dermatologue et un cosmetologue) ont évalué les photos pour l'effet sur plusieurs caracteristiques faciales cosmétiques. Les taies d'oreiller contiennent le cuivre ont eu un effet positif pour les caracteristiques faciales suivantes: la réduction de rides ($P < 0.001$) and et pattes d'oie/lignes fines ($P < 0.001$) et l'amélioration de l'apparence generale ($P < 0.001$), au point de 2 et 4 semaines. Le sommeil sur les taies d'oreiller contiennent l'oxide

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de cuivre a causé une réduction significative dans l'apparence de rides faciales et pattes d'oie/lignes fines et une amélioration significative dans l'apparence de la peau faciale. Dans la plupart des participants, cet effet était déjà visible dans 2 semaines après l'utilisation des taies d'oreiller contenant l'oxyde de cuivre.

Introduction

Copper is an essential trace element involved in numerous human physiological and metabolic processes [1], including skin formation [2, 3] and wound repair [4]. The recommended daily allowance of copper is 0.9 mg [5]. Copper is safe to humans as demonstrated by the widespread and prolonged use of copper intrauterine devices [6] and over-the-counter wound healing treatments containing copper [7, 8]. The risk of adverse reactions as a result of dermal contact with copper is extremely low [9]. A Phase I clinical trial found that applications of ointment preparations containing copper in concentrations up to 20% did not cause any adverse reactions or toxicity [10]. The concentrations of copper found in the serum of the 61 volunteers who participated in this study augmented indicating that copper can be absorbed through the facial skin [10]. The capacity of copper to penetrate the human stratum corneum was confirmed in a separate study [11].

A durable platform technology was developed, which introduces copper oxide to textile fibres [12, 13]. The copper-impregnated textiles possess a broad spectrum of antimicrobial and antifungal properties, without causing skin sensitization or irritation [12–14]. The effectiveness of treating athlete's foot infections by using copper oxide

containing socks has been recently demonstrated [14]. Interestingly, it was observed that the overall appearance of the skin of individuals using these socks, most of whom were elderly people, was much healthier, smoother and aesthetically improved. These benefits were also found in the non-infected skin areas (Zatcoff R.C., unpublished results).

Based on the above observation on the role that copper plays in skin regeneration (see Results and Discussion) and on the capacity of facial skin to absorb copper [10], we hypothesized that sleeping on pillowcases containing copper would have a significant positive cosmetic effect on the individuals using them. In the present study we demonstrate in a double-blind placebo-controlled study that sleeping on copper-containing pillowcases has a positive effect on several facial cosmetic skin characteristics.

Materials and methods

Test items

Two test items (TI) were included in the study: a cotton/polyester pillowcase containing 0.4% (weight/weight) copper oxide particles (TI1; Fig. 1) and a placebo control cotton/polyester pillowcase (TI2) not containing copper oxide. There were no differences in the construction, colour, structure and feel of the two TIs.

Test participants

Fifty-eight healthy volunteers (56 women and 2 men), aged 40–60 years, who received a score between 2 and 4 on a 5-point scale (1 = no wrinkles, 5 = severe wrinkling) were enrolled in the

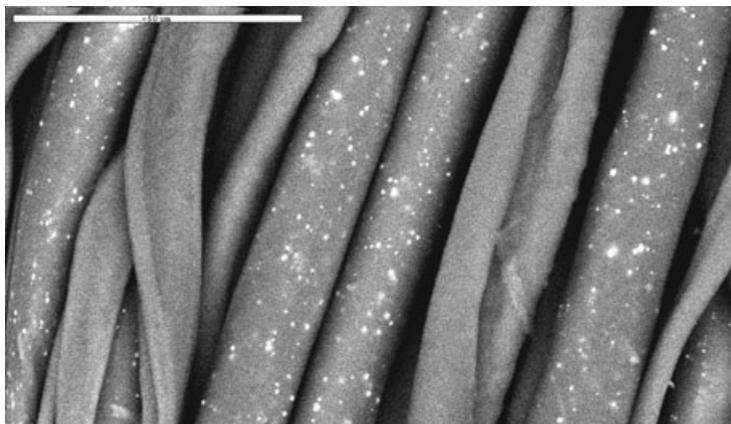


Figure 1 Scanning electronic microscope image of the cotton/polyester fibre blend used in the copper-containing pillowcases. The white dots are the copper oxide particles embedded in the polyester fibres.

study. One trial participant dropped out for personal reasons before completion of the trial. All participants signed an informed consent form and the trial was approved by the Kaplan Medical Center Ethics Committee.

Study outline

All volunteers were given a code number and randomly assigned to Group A or B. Photographs of the front and both sides of the face were taken at Day 0 (Week 0), Day 14 (Week 2) and Day 28 (Week 4) by a professional photographer. Constant lighting and photographic conditions were maintained during all photography sessions. The volunteers were seated on a high chair and requested to first look directly into the camera (pictures were taken of the front of the face) and then 90° to the left (pictures were taken of the right side of the face) or 90° to the right (pictures were taken of the left side of the face) to pre-marked spots on the relevant wall at ~1.2 m above the floor. Each photo was marked with the participant's code number, area of the face photographed (front, left, or right) and the date the photo was taken. On Day 0, trial participants were given two pillowcases, either TI1 or TI2, according to the group to which they were randomly assigned. Participants were instructed to use only pillowcases given to them and not their regular pillowcases during the course of the study and to use (wash, store, etc.) the TI in the same way as they would use their regular pillowcases but without using a fabric softener during the washing or drying cycle. In addition, they were instructed not to use moisturizers or other facial creams on their face at night throughout the 4 weeks trial period.

The photographs were examined by two expert graders (Anna Lyakhovitsky, MD, Dermatologist and Natasha Kalinkovich, Cosmetician) at the end of 4 weeks by comparing the front photos taken at Weeks 0, 2 and 4 side-by-side, then by comparing the left side photos taken at Weeks 0, 2 and 4 side-by-side and finally by comparing the right side photos taken at Week 0, 2 and 4 side by side, for each of the participants. A score for each of the cosmetic facial skin characteristics was given using standard cosmetic/dermatological scores [15, 16] of 1: Absent; 2: Mild; 3: Moderate; 4: Severe; and 5: Extreme. The following cosmetic characteristics were scored: wrinkles; crow's feet/fine lines; blemishes; skin glow (defined as 'shining skin'); and

general appearance (defined as 'skin that appeared generally healthy and without damage and wear-and-tear because of the ravages of time, sun exposure, etc.').

Data analysis

Data analysis was performed by MediStat Ltd. (Statistics in Medicine, Tel Aviv, Israel). One of the trial participants did not complete the trial for personal reasons. Her data were not included in the analysis. A total of 108 scores (6 facial skin attributes \times 3 photos of the face \times 3 times) per trial participant was given by each grader. For all facial skin attributes, a numerically decreasing score (i.e. from 3 to 2 or from 4 to 1) was considered as an improvement in that facial skin characteristic. When the movement was 1 on the scale, a grade of 1 was given (i.e. from 3 to 2 or from 2 to 1). When the movement was 2 on the scale, a grade of 2 was given (i.e. from 3 to 1 or from 4 to 2). No movement along the scale received a score of 0. A numerical increase in the scale (deterioration) received a correspondingly negative score.

Statistical Wilcoxon scores (rank sums) and chi-squared tests were used to compare between expert grader scores for TI1 and TI2 at baseline Week 0 and to compare the differences at Week 2 and Week 4, respectively, from the baseline (i.e. Week 2 vs. Week 0 and Week 4 vs. Week 0 per each examined facial skin characteristic).

Results and Discussion

Table I summarizes the mean \pm SE for the changes from baseline obtained for the Control and Treatment groups for the right side, left side or front pictures alone, or when combined together (defined as Total). When pooling the scores given for the front and both sides of the faces, there was a statistically significant reduction of the appearance of facial wrinkles and crow's feet/fine lines and statistically significant improvement of overall appearance of facial skin at Week 2 and Week 4 when compared with the baseline (Week 0) of the participants who used the copper-containing pillowcases. Figure 2 shows representative examples of the side and front pictures taken from three participants using the copper oxide containing pillowcases. When analyzing the data for the right side, left side or front pictures alone (i.e. without combining the data), in most cases a statistical

Table I Mean \pm SE of changes from baseline (week 0) and Wilcoxon scores (rank sums) and chi-squared for Wilcoxon comparison between groups for changes from baseline (week 0)

	2 Weeks			4 Weeks				
	Front	Right	Left	Total	Front	Right	Left	Total
Wrinkles								
Control	0.0741 \pm 0.07	0.148 \pm 0.0878	0.111 \pm 0.0616	0.1059 \pm 0.0411	0.11 \pm 0.0815	0.1481 \pm 0.0697	0.148 \pm 0.0697	0.1294 \pm 0.0403
Treatment	0.233 \pm 0.078	0.3214 \pm 0.09	0.3571 \pm 0.0922	0.306 \pm 0.0503	0.2 \pm 0.0743	0.4643 \pm 0.1089	0.428 \pm 0.0952	0.3647 \pm 0.0551
P value ¹	0.0500*	0.0860	0.0390*	0.0005*	0.2129	0.0258*	0.0280*	0.0005*
P value ²	0.0331*	0.0590	0.0318*	0.0002*	0.1717	0.0221*	0.0221*	0.0003*
Fine lines								
Control	0.2083 \pm 0.0847	0.125 \pm 0.069	0.0417 \pm 0.0417	0.125 \pm 0.0392	0.1538 \pm 0.0722	0.1154 \pm 0.0639	0.1923 \pm 0.0788	0.1538 \pm 0.0411
Treatment	0.428 \pm 0.0952	0.3571 \pm 0.0922	0.3929 \pm 0.094	0.3929 \pm 0.0536	0.3333 \pm 0.0875	0.6 \pm 0.1292	0.5 \pm 0.115	0.4778 \pm 0.0637
P value ¹	0.1030	0.0636	0.0047*	0.0003*	0.1337	0.0031*	0.0521	0.0002*
P value ²	0.0915	0.0539	0.0027*	0.0002*	0.1219	0.0021*	0.0541	0.0001*
Blemishes								
Control	0.0 \pm 0	0.0 \pm 0	0.04 \pm 0.04	0.013 \pm 0.0133	0.0385 \pm 0.0385	0.0769 \pm 0.0533	0.125 \pm 0.069	0.0789 \pm 0.0311
Treatment	0.0357 \pm 0.0357	0.0 \pm 0	0.0357 \pm 0.0357	0.0238 \pm 0.0167	0.1 \pm 0.0557	0.1 \pm 0.0557	0.2 \pm 0.0743	0.1333 \pm 0.036
P value ¹	0.3685	1.0000	0.9572	0.6349	0.3906	0.7785	0.4780	0.2659
P value ²	0.3401	0.988	0.9349	0.6279	0.3725	0.7626	0.4624	0.2615
Glow								
Control	0.24 \pm 0.0872	0.16 \pm 0.0748	0.12 \pm 0.0663	0.1733 \pm 0.044	0.2692 \pm 0.0887	0.231 \pm 0.0843	0.2308 \pm 0.1	0.2436 \pm 0.0522
Treatment	0.2222 \pm 0.0815	0.1429 \pm 0.0673	0.1429 \pm 0.0673	0.1687 \pm 0.0414	0.3667 \pm 0.1015	0.3667 \pm 0.1123	0.2333 \pm 0.078	0.3222 \pm 0.0566
P value ¹	0.8908	0.8751	0.8207	0.9405	0.5674	0.4918	0.7830	0.3668
P value ²	0.8792	0.8618	0.8062	0.9381	0.6028	0.5596	0.7090	0.6028
Overall appearance								
Control	0.0417 \pm 0.0417	0.0417 \pm 0.0417	0.0417 \pm 0.0417	0.0417 \pm 0.0237	0.1154 \pm 0.0639	0.1154 \pm 0.0639	0.1538 \pm 0.0722	0.1282 \pm 0.0381
Treatment	0.2143 \pm 0.079	0.2857 \pm 0.0869	0.2143 \pm 0.079	0.2381 \pm 0.0468	0.3667 \pm 0.0895	0.5 \pm 0.0928	0.4333 \pm 0.092	0.4333 \pm 0.0525
P value ¹	0.0802	0.0267*	0.0802	0.0008*	0.0372*	0.0037*	0.0293*	<0.0001*
P value ²	0.0690	0.0204*	0.0690	0.0006*	0.0303*	0.0021*	0.0233*	<0.0001*

¹Wilcoxon scores.²Chi-squared test.* $P \leq 0.05$.

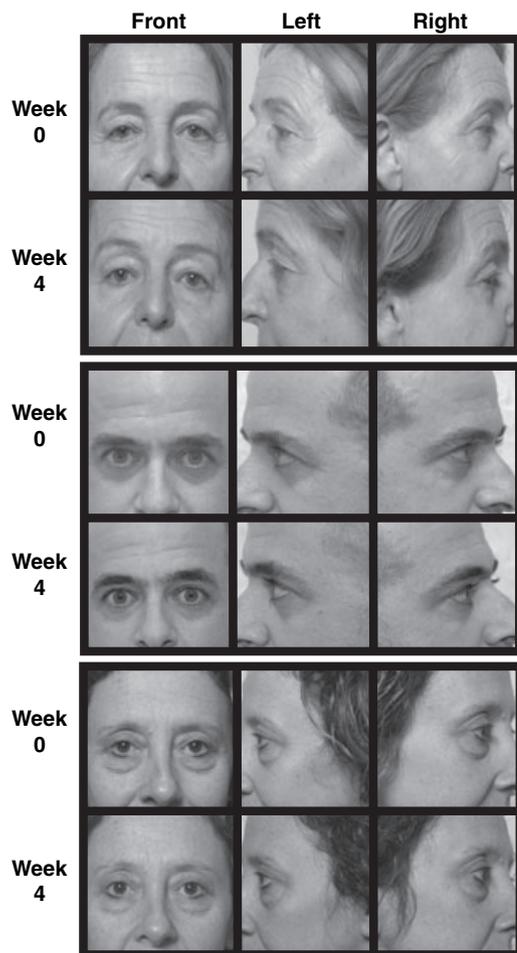


Figure 2 Representative pictures showing the reduction of wrinkles and fine lines and improvement of overall appearance of the facial skin of individuals sleeping on the copper-containing pillowcases.

significance was achieved ($P < 0.05$) for the reduction in the appearance of facial wrinkles and crow's feet/fine lines and improvement of the overall appearance of facial skin, both at Week 2 and Week 4. However, the statistical strength (P value) was lower than when examining the pool of data. This is explained mainly by the significantly lower number of data points included in the analysis. A possible explanation that was postulated was that some individuals sleep mainly on one side of their face during the night. However, most participants responded that they sleep on both sides of their faces during the night.

Statistically significant results were obtained when using either the Wilcoxon scores (rank sums) or the chi-squared tests, both being appro-

prate tests to analyse the data. This further strengthened the conclusion that sleeping on pillowcases containing at least 0.4% weight/weight copper oxide particles had a positive cosmetic effect on several cosmetic characteristics such as reducing the appearance of wrinkles and fine lines and improving overall skin appearance. Similar results were obtained in a similar study conducted by an independent cosmetic company with 64 women; however, the details of this study could not be included in this report because of a confidentiality agreement.

Not a single adverse reaction was noted during the study. This is in accordance to the very low risk of any adverse reaction because of dermal contact with copper [9]. Furthermore, at the end of the trial, all trial participants were given two pillowcases containing copper oxide as a gift. Six months later they were given a questionnaire and asked if they used the pillowcases, if so, how frequently and whether they had experienced any adverse reactions. Most participants used the pillowcases regularly for 2–4 weeks per month without experiencing any negative reaction (data not shown), either dermal or other.

A possible concern may be the inhalation of copper oxide particles. However, in an experiment using good laboratory practices (GLP) conducted by an independent laboratory (Nelson Laboratories, Salt Lake City, UT, U.S.A.), human inhalation conditions were simulated for 5 h and the amount of copper particles released from copper oxide impregnated fabrics containing five times the amount of copper oxide in the copper oxide containing pillowcases [i.e. in fabrics containing 2% (weight/weight) copper oxide particles] was determined. The amount of copper particles measured was negligible and well below permissible exposure limits for copper (data not shown) according to the United States Occupational Safety and Health Administration (OSHA), which indicated that pillowcases containing 0.4% copper oxide (w/w) did 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 from a fabric impregnated with five times the amount of copper found in the tested copper-containing pillowcases into

simulated saliva was determined. This experiment simulated an extremely unlikely scenario whereby secreted saliva coming into contact with the pillowcases during sleep might be ingested. The amount of copper that eluted into the saliva was below the minimal risk level (MRL) for copper oral exposure and determined to be $0.01 \text{ mg kg}^{-1} \text{ day}^{-1}$ by the Agency for Toxic Substances and Disease Registry of U.S. Department of Health and Human Services, indicating that even a fabric with five times as much copper as that used in the study would not pose a risk because of possible consumption of the copper that might elute into saliva.

Why does sleeping on a copper-containing pillowcase have a positive effect on the skin? A possible physiological explanation in the reduction in wrinkles and fine lines and improvement of the overall appearance when sleeping on the copper-containing pillowcases may be that the copper ions are liberated into the moisture found between the face and the pillowcase. These ions are then absorbed through the skin. Once absorbed they stabilize and stimulate the formation of extracellular matrix proteins, such as collagen, fibronectin and integrin. This is in accordance with the following observations: i) absorption of copper or copper oxide applied on the facial skin has been demonstrated [10]; ii) requirement of copper as a cofactor for efficient extracellular matrix protein cross-linking, including of collagen, elastin and fibronectin, by several proteins such as lysyl oxidase [3, 17, 18]; iii) reduced collagen formation and lysyl oxidase activity [19, 20] in Menkes patients; iv) presence of the copper-dependant superoxide dismutase enzyme, important in protection against free radicals, in the skin [21]; v) increase in stabilization of fibronectin mats [22, 23] and collagen cross-linking by copper ions [24]; vi) modulation of integrins expression by keratinocytes [25] induced by copper; vii) increase in protein synthesis of collagen and elastin by interaction of copper with human peptide Gly-(L-His)-(L-Lys) or GHK [2]; and viii) acceleration of epithelial tissue growth [26] by topical copper sulphate treatment. Accordingly, several commercial facial creams contain copper as their active ingredient (e.g. Neutrogena Visibly Firm® Face Lotion, SPF 20: Neutrogena, Los Angeles, CA, USA). In conclusion, this study confirms our hypothesis that sleeping on fabrics that liberate copper ions would have a significant positive cosmetic effect on the individuals using them.

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