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ORIGINAL PAPER

Effect of oral intake of choline-stabilized orthosilicic acid on hair tensile strength and morphology in women with fine hair

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Received: 8 May 2007 / Revised: 20 August 2007 / Accepted: 30 September 2007 / Published online: 25 October 2007 © Springer-Verlag 2007

Abstract The appearance of hair plays an important role in people's overall physical appearance and self-perception. Silicon (Si) has been suggested to have a role in the formation of connective tissue and is present at 1–10 ppm in hair. Choline-stabilized orthosilicic acid ("ch-OSA") is a bioavailable form of silicon which was found to improve skin microrelief and skin mechanical properties in women with photoaged skin. The effect of ch-OSA on hair was investigated in a randomized, double blind, placebo-controlled study. Forty-eight women with fine hair were given 10 mg Si/day in the form of ch-OSA beadlets (n = 24) or a placebo (n = 24), orally for 9 months. Hair morphology and tensile properties were evaluated before and after treatment. Urinary silicon concentration increased significantly in the ch-OSA supplemented group but not in the placebo group. The elastic gradient decreased in both groups but the change was significantly smaller in the ch-OSA group (-4.52%)compared to placebo group (-11.9%). Break load changed significantly in the placebo group (-10.8%) but not in the ch-OSA supplemented group (-2.20%). Break stress and

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N. Demeester · D. Vanden Berghe · M. Calomme (⊠) Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Antwerp, Belgium e-mail: microfar@ua.ac.be elastic modulus decreased in both groups but the change was smaller in the ch-OSA group. The cross sectional area increased significantly after 9 months compared to baseline in ch-OSA supplemented subjects but not in the placebo group. The change in urinary silicon excretion was significantly correlated with the change in cross sectional area. Oral intake of ch-OSA had a positive effect on tensile strength including elasticity and break load and resulted in thicker hair.

Keywords Hair · Tensile strength · Choline-stabilized orthosilicic acid · Elasticity · Break load

Introduction

Hair is undoubtedly one of the most important attributes of people in all cultures, and thus its properties are of great psychological importance. Human hair fibers consist of a sulfur-rich outer protective cuticle layer surrounding highly keratinized cortex cells. The structural hierarchy and disulphide cross-linking nature of keratins results in the remarkable mechanical properties of hair fibers [19, 20, 36, 37]. These mechanical properties (i.e. tensile strength, elasticity) can be altered by external factors including UV irradiation and sunlight [5, 22], moisture and temperature [3]. Disturbances in hair growth and quality can also be induced by protein malnutrition or suboptimal intake of trace elements and vitamins [30]. These deleterious effects are manifested to the consumer in the form of decreased manageability, brittleness and dryness.

Silicon (Si) is a ubiquitous element present in various tissues in the human body [1] and is present at 1–10 ppm in hair [31] and nails [11]. Possibly, silicon accumulates in the cornified epidermis and in the epicuticle of hair [11, 21] as

was demonstrated in older studies. Dietary silicon deficiency in growing animals indicated growth retardation and marked defects of bone and connective tissue [13], most likely due to decreased collagen and glycosaminoglycan synthesis [11]. In vitro, the activity of prolyl hydroxylase was reported to be dependent on the Si concentration in the medium of bone cultures, suggesting a Si-dependent pathway for collagen type I synthesis [12]. Others have suggested a structural role of Si in the cross-linking of glycosaminoglycans in connective tissue [29].

Silicon is present in beverages and water in the form of orthosilicic acid (OSA). OSA is stable in dilute concentrations ($<10^{-4}$ M) but polymerizes at higher concentrations into a range of silica species. Absorption studies indicated that only orthosilicic acid is bioavailable whereas its polymers are not absorbed [24]. Dietary silicates undergo hydrolysis, forming orthosilicic acid, which is readily absorbed in the gastrointestinal tract. Physiological concentrations of orthosilicic acid were recently found to stimulate the synthesis of collagen type I in skin fibroblasts [27].

A stabilized form of orthosilicic acid, choline-stabilized OSA ("ch-OSA"), was found to have a high bioavailability in humans compared to polymerized forms of OSA [8, 35]. Supplementation of animals with low doses of ch-OSA complex resulted in a higher collagen concentration in the skin [7] and in an increased femoral bone density [9, 10]. Oral intake of ch-OSA during 20 weeks in women with photoaged skin resulted in a significant positive effect on skin surface and skin mechanical properties [2], suggesting a regeneration or de novo synthesis of collagen fibers. Assessment of hair brittleness on a visual analogue scale (VAS) also indicated an improvement in ch-OSA supplemented subjects compared to the placebo group.

In the present study, we investigated the effect of oral intake of choline-stabilized OSA ("ch-OSA") on hair tensile strength and morphology in a randomized, placebocontrolled double blind study.

Subjects and methods

Subjects

Forty-eight healthy Caucasian females, aged between 18 and 65 years, with fine hair and a sufficient hair length (minimum 15 cm) to permit tensile strength measurements were included in this study. The assessment of hair during screening was evaluated by a professional hairdresser on a visual analogue scale (3-point scale: fine, normal and thick). Only women with a score "fine" were allowed to participate in the study. All the women gave written informed consent. Women, using silicon supplements less than 3 months before the start of the trial or any food supplement other than the study medication during the trial were excluded. Subjects with a known allergy to one of the ingredients of the study medication (i.e. stabilized orthosilicic acid, choline chloride, microcrystalline cellulose) were not allowed to participate. In addition, subjects with colored and/or permed hair were excluded. Furthermore, chemical treatment of the hair such as perming, coloring or bleaching and intake of pharmaceuticals (i.e. vitamin supplements, anticoagulants, systemic antibiotics, heparin, retinoids) that could interfere with the outcome of the study was prohibited during the trial. Subjects were asked not to change daily hair care during the study and had a hair cut every 4 weeks at the research institute (Institute Dr. Schrader, Holzminden, Germany) by the same hairdresser. Subjects with diseases that could influence hair parameters, such as psoriasis and folliculitis, were excluded. Participation in another clinical trial was prohibited. The trial was started in the autumn of 2004 and was completed in the summer of 2005.

Ethical approval was obtained from the regional Ethics Committee (Ethikkommission bei der Ärztekammer Niedersachsen, Hannover, Germany, protocol number 04/1). The study was carried out in accordance with the Declaration of Helsinki (1964) changed by the 29th World Medical Assembly at Tokyo (1975).

Study medication

Subjects were randomly assigned to two groups to take two capsules daily containing either a placebo (microcrystalline cellulose beadlets, Pharmatrans Sanaq AG, Switzerland) or 10 mg of silicon in the form of ch-OSA beadlets (Bio Minerals n.v., Belgium) over a 9 month period. Subjects were instructed to take one capsule in the morning and another capsule in the evening with a glass of water or juice. Placebo and ch-OSA capsules were identical in color, taste, odor and packaging.

Patient compliance was assessed at each visit by quantifying the amount of study medication returned. Patients and investigative site staff were blinded to the group assignment throughout the study.

Urine analysis

Single void urine samples were collected from fasting subjects at baseline and after 9 months supplementation, using Si-free polypropylene tubes (Sarstedt, Germany). Si concentration was analyzed in one batch by electrothermal atomic absorption spectrometry with inverse longitudinal Zeeman background correction (AAnalyst 800, Perkin Elmer, Bodenseewerk, Germany). Pyrolytic coated graphite tubes were used. The hollow cathode lamp settings were: 30 mA lamp current, 251.6 nm spectral line and 0.2 nm band width. The injected sample volume was 20 µl and signals were measured in the peak–area mode. Samples were measured in duplicate by standard addition. Standards and urine dilutions were prepared in matrix modifier solution containing 72 mg/l CaCl₂ (Aldrich, Belgium), 1.508 g/l NH₄H₂PO₄ (Merck, Belgium) and 0.5 g/l Na₄EDTA (Aldrich, Belgium) in ultrapure water (conductance $\leq 0.08 \,\mu$ S). The sensitivity, determined as the amount of silicon yielding a 0.0044 absorbance signal was 90 pg. Safety parameters such as the concentration of glucose, proteins, ketones, bilirubin, urobilinogene, blood, nitrite, leukocyte esterase, pH, urea, uric acid, creatine, sodium, potassium, calcium, phosphor and magnesium were measured at baseline and after 9 months supplementation.

Evaluation of hair quality: tensile strength and morphology properties

Tensile strength of hair samples (100 single hair fibers per sample) was measured using a MTT 170/670 Series Miniature Tensile Tester (Diastron Ltd, UK). Each hair was fixed between two ferrules and placed in the sample cassette of the instrument. For each hair sample, the first ferrule was placed at 3 cm from the root end and the distance between both ferrules was also 3 cm. The instrument exerts a constant speed of extension (extension rate: 20 mm/min) on a single hair fiber and extends the fiber until it breaks. The pre-gauge is 19.6131×10^{-3} N for each sample. The measurement is automatically terminated when a fracture is detected (break point) or when the force limit of the instrument (maximum force: 1.96133 N) is achieved. Break stress (break load/cross sectional area) and elastic modulus (elastic gradient/cross sectional area) was calculated using average values (100 hairs/sample/subject) of apparent diameter, cross-sectional area [(apparent diameter/2)² $\times \pi$], break load and elastic gradient, respectively. All measurements and evaluations were made in an air-conditioned laboratory to guarantee a constant room temperature of 22°C $(\pm 1^{\circ}C)$ and 50% $(\pm 5\%)$ relative humidity.

A hair sample (400–500 hairs) was taken from the back of the head (occipital) at an identical area at baseline and after 9 months supplementation. Part of the sample was used for tensile strength measurements and another part for measuring minor and major axis.

Tensile strength measurements were performed at baseline, and after 9 months of supplementation. The study started in the autumn of 2004 (between October 28 and November 17) and was completed in the summer of 2005 (between July 15 and August 8). At baseline, the tensile strength of hair formed during the previous months was measured, i.e. from June till October 2004. After 9 months of supplementation, the tensile strength of hair formed from March till July 2005 was measured. A climatological database (http://www.wetteronline.de, data recorded at Beverungen which is located 20 km from the clinical centre in Holzminden, Germany) was used to assess if seasonal differences in temperature, hours of sun and relative humidity could have influenced the observed tensile strength results.

The cross-sectional area was determined according to an experimental procedure developed by Teasdale et al. [34]. A bundle of about a 100 hairs was placed in a thin shrinkage tube. This sample consists of hairs that are not identical to the hairs used for tensile strength measurements, but consists of hair derived from the same head area. The jutting ends of the hairs were mechanically stretched during shrinkage of the tubes by heating. The tube was cut perpendicularly to its length and the fibre ends were embedded in an ether-starch solution (colloidon 4%) by dipping the tube into the solution. Sections of 100-200 µm thickness were cut, depending on the intensity of the pigmentation, i.e. thinner slices are cut for intense pigmented hair. The slices were fixed on a slide and investigated by light microscopy (ca. $600 \times$ magnification). Major and minor axes were measured and the cross sectional area was calculated. The cross sectional area was calculated as follows: [(minor axis \times major axis/2)² $\times \pi$]. The apparent diameter was calculated as the square root of (minor axis \times major axis).

Statistical analysis

SPSS software (version 13.0, Chicago, USA) for Windows was used for statistical analysis. Non-parametric tests were used since a one-sample Kolmogorov–Smirnov test indicated that data were not normally distributed.

Differences between groups were evaluated with a Mann–Whitney U test and differences within groups were analyzed with a Wilcoxon matched-pairs signed rank test. The relation between two parameters was investigated with the Spearman correlation procedure. All statistical tests were two-sided and considered statistically significant at P < 0.05.

Results

The study was conducted in 48 women with fine hair. Of 48 eligible women randomized into the study, 45 completed the study (placebo: 23 subjects, ch-OSA: 22 subjects) with acceptable compliance (placebo group: $98.4 \pm 2.33\%$, ch-OSA group: $98.3 \pm 4.40\%$). Reasons for withdrawal were suspected pregnancy or volunteer decision (non-medical). One case of thrombosis in the cerebellum (ch-OSA group) was classified by the ethical committee as a serious adverse event unrelated to the study medication, considering baseline safety parameters and the specific pathology.

Table 1 Baseline characteristics of patients (n = 45), mean \pm SD

	Placebo	ch-OSA
Group characteristics		
Total number	<i>n</i> = 23	<i>n</i> = 22
Body mass index	24.7 ± 4.93	24.7 ± 6.02
Age	42.5 ± 2.42	44.1 ± 2.43
Si in urine (µg/µmol creatinine)	0.58 ± 0.23	0.48 ± 0.20
Hair characteristics		
Hair color		
White (<i>n</i>)	1	0
Blonde (<i>n</i>)	5	3
Medium blonde (<i>n</i>)	8	9
Dark blonde (<i>n</i>)	5	4
Brown (<i>n</i>)	3	5
Dark brown (<i>n</i>)	1	1
Morphology parameters		
Minor axis (µm)	49.4 ± 4.38	51.6 ± 3.66
Major axis (µm)	69.8 ± 9.55	72.5 ± 7.53
Cross sectional area (mm ² \times 10 ⁻³)	2.71 ± 0.56	2.94 ± 0.46
Tensile strength parameters		
Elastic gradient (N % ⁻¹)	0.09 ± 0.02	0.09 ± 0.01
Elastic modulus (N m ^{-2} × 10 ⁹)	3.31 ± 0.39	3.09 ± 0.32
Yield extension (%)	26.6 ± 1.73	27.1 ± 1.20
Break load (N)	0.69 ± 0.16	0.70 ± 0.11
Break stress (N m ^{-2} × 10 ⁶)	254 ± 33.7	241 ± 20.6

Baseline characteristics are presented in Table 1. Mean values for age (placebo group: 42.5 ± 2.42 years; ch-OSA group: 44.1 ± 2.43 years), body mass index (placebo group: 24.7 ± 4.93 ; ch-OSA group: 24.7 ± 6.02), and the different hair parameters were not significantly different between the two groups. The mean apparent diameter was (placebo: 58.5 ± 6.0 ; ch-OSA: 61.0 ± 4.9) almost identical in both the groups to the threshold value of thin hair

Break load (N)

Break stress (N m⁻² x 10⁶)

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 $(60 \ \mu m)$, which was previously specified by Zviak [39]. It should however be noted that no generally accepted threshold values exist which define "fine" hair.

Urine analysis

Biochemical safety parameters were analyzed in urine at baseline and after 9 months treatment. All urine parameters were within the normal range at baseline and after 9 months supplementation in both groups.

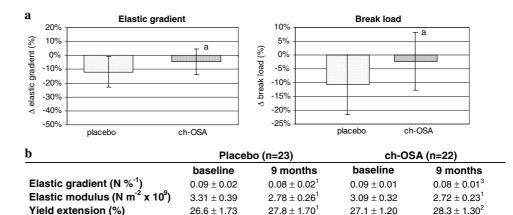
The mean urine Si concentration was not significantly different at baseline (placebo: 0.58 µg Si/µmol creatinine; ch-OSA: 0.48 µg Si/µmol creatinine) and increased significantly after 9 months in the ch-OSA group (0.76 µg Si/µmol creatinine, P < 0.05) but not in the placebo group (0.70 µg Si/µmol creatinine, NS).

Tensile strength measurements

In order to evaluate the tensile strength, hair extension measurements were performed at baseline and after 9 months supplementation. The following characteristic parameters were determined: mean gradient of elastic phase (elastic gradient), modulus of elasticity in the linear region, extension of the yield phase (yield extension), post yield phase gradient increase resulting in fracture of the fiber (break load) and break load correlated to area (break stress) (Fig. 1).

The elastic gradient decreased in both groups but the change was significantly smaller in the ch-OSA group (-4.52%, P = 0.027) compared to the placebo group (-11.9%), (Fig. 1b). The yield extension increased significantly in both groups and the observed change was comparable for both groups, i.e. placebo 4.33% and ch-OSA 4.57%. Compared to baseline, the break load decreased significantly after 9 months in the placebo group but not in the ch-OSA group. The change in break load was significantly

Fig. 1 a Change (%) in tensile strength parameters after 9 months supplementation compared to baseline (mean \pm SD, %). **b** Tensile strength parameter values at baseline (T0) and after 9 months (T9) supplementation. *a* P < 0.05 (Mann–Whitney *U* test) versus placebo; (1) P < 0.0001, (2) P < 0.005, and (3) P < 0.05 (Wilcoxon test) versus baseline



 0.61 ± 0.13^{1}

216 ± 19.6

 0.70 ± 0.11

 240 ± 20.6

 0.69 ± 0.12

 217 ± 17.6^2

 0.69 ± 0.16

 254 ± 33.7

smaller in the ch-OSA group (-2.20%) compared to placebo (-10.8%, P = 0.011). The break stress decreased significantly after 9 months supplementation in both groups, but the observed change tended to be smaller in the ch-OSA group (-9.41%) compared to the placebo group (-14.2%, P = 0.063).

Baseline load-elongation (stress-strain) curves (data not shown) were almost identical for both groups, which confirm similar hair characteristics of subjects in these groups. After 9 months, the break point is shifted to a lower force in both groups but the observed decrease is significantly smaller in the ch-OSA group.

Hair morphology measurements

Several morphology parameters were determined at baseline and after 9 months supplementation including crosssectional area, minor axis and major axis.

After 9 months supplementation, the cross-sectional area was significantly increased in the ch-OSA group but not in the placebo group, compared to baseline. The increase in minor and major axis and the cross section was more pronounced in the ch-OSA group compared to the placebo group (Fig. 2).

The baseline cross-sectional area correlated strongly with the elastic gradient and the break load but not with break stress (Table 2). Interestingly, the change in Si (baseline versus 9 months) excretion was positively correlated with the change in cross-sectional area (r = 0.355, P = 0.023, data not shown).

Discussion

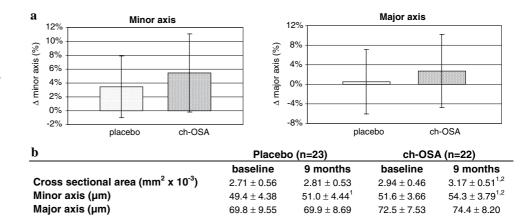
We previously demonstrated that oral intake of low doses of ch-OSA (5% increase of total dietary Si intake) during 24 weeks in calves resulted in a significantly higher hydroxyproline content in the dermis compared to placebo, **Table 2** Spearman correlation between cross sectional area and tensile strength parameters in the total study population (n = 45) at baseline

	Cross sectional are	ea
Tensile strength	r	Р
Elastic gradient	0.756	< 0.001
Break load	0.752	< 0.001
Break stress	-0.195	NS

r correlation factor

and also found a significant correlation between the serum Si concentration and the hydroxyproline content in cartilage [7]. Hydroxyproline is a specific and major amino acid in collagen. Reffitt et al. [27] found that low levels of orthosilicic acid (typical serum concentrations), stimulate the synthesis of collagen type I in cultures of human osteoblasts and skin fibroblasts. The orthosilicic acid-dependent stimulation of collagen synthesis was abolished in the presence of prolyl hydroxylase inhibitors. As type I collagen and its monomer hydroxyproline are major constituents of skin, the improvement in skin parameters after ch-OSA supplementation points to potential regeneration or de novo synthesis of collagen fibers. Silicon was also reported to be involved in the synthesis of glycosaminoglycans [29] and was suggested to have a structural role as a cross-linking agent in connective tissue. Accordingly, treatment with ch-OSA might improve the glycosaminoglycan structure in the dermis and the keratin structure in hair and nails. Furthermore, the choline compound present in ch-OSA might have a synergistic effect with orthosilicic acid since it is well known that choline is involved in several basic biological processes [4], including the fact that choline is a precursor of phospholipids such as phosphatidyl choline which is an essential component of cellular membranes. The physiological significance of choline is substantiated by the fact that intentional deprivation of choline disrupts cell growth and division [38].

Fig. 2 a Change (%) in hair morphology parameters after 9 months supplementation compared to baseline (mean \pm SD, %). **b** Mean values \pm SD of hair morphology parameters at baseline (T0) and after 9 months (T9) supplementation. (1) *P* < 0.05 (Wilcoxon test) versus T0; (2) *P* < 0.05 (Mann–Whitney *U* test) versus 9 months placebo



The present study is the first to our knowledge of a randomized, double blind and placebo-controlled study that illustrates a positive effect of an oral supplement on hair quality parameters in women. The dose of ch-OSA supplementation (10 mg Si/day) was low compared to the average daily Si intake of 20–50 mg as reported previously by Pennington [25]. The major dietary sources of Si are cereal/ grain-based products and vegetables but modern food processing, including refining, is likely to reduce the dietary Si intake as it was shown that fibers contribute the most to the silicon content in plant based foods [32]. The intake of 10 mg Si in the form of ch-OSA is safe as no adverse effects related to the study medication were reported. Furthermore, urine safety parameters remained within the normal range after ch-OSA supplementation.

A decrease in elastic gradient was observed in both groups after 9 months of supplementation but the change was significantly smaller in the ch-OSA group. Elasticity is one of the most important properties of hair. Because of elasticity, hair can resist forces beyond certain limits without resulting in permanent damage, i.e. the hair will regain its original length when the stress disappears. Hair with poor elasticity will stretch only to a limited extent. Additional stress will cause permanent deformation or breaking. Our data indicate that the loss of elasticity is reduced by ch-OSA supplementation.

Break load decreased in the placebo group, whereas in the ch-OSA group only a mild decrease was observed. Accordingly, break stress (break load/area) decreased in both groups, but the change was smaller in the ch-OSA group compared to the placebo group. Other studies [16, 26] have reported seasonal changes in hair growth and shedding of hair, i.e. Courtois et al. [16] indicated an overall annual periodicity, manifested by a maximal proportion of telogen hairs at the end of the summer and the beginning of autumn. We did not find studies documenting a seasonal change in tensile strength. On the other hand, deleterious effects of sunlight and UV radiation on tensile strength are well documented [28]. It is widely accepted that exposure to sunlight and UV initiates photodegradation processes that lead to irreversible chemical and physical changes to the hair. Since baseline tensile strength measurements involved hairs formed during June-October 2004 and was repeated 9 months later on hairs formed during March-July 2005, we examined whether these changes could be explained by a seasonal shift in temperature and/or relative humidity. Climatological data collected from an onlinedatabank (http://www.wetteronline.de) showed a difference between the two periods of 25 h of sun (495 vs. 520 h), 66.7 mm of rain (240 mm vs. 173 mm) and a comparable relative humidity. Consequently, these moderate seasonal differences cannot explain the loss in tensile strength that is observed in the placebo group. Possibly the two periods differ in the total amount of time that subjects spend outside, i.e. perhaps in the second period the subjects had more outdoor activities and were more exposed to the sun and/or higher temperatures resulting in more pronounced photo-ageing. However, this remains purely hypothetical, as indoor and outdoor activities of subjects were not recorded in this study. A detailed study of seasonal change of tensile strength was not the aim of the present intervention study and should be confirmed by other studies.

Several models were described by others to explain tensile strength properties of hair, including the transition of α keratin to β -keratin when hair is stretched. Cysteine crosslinkages, coulombic interactions between side chain groups, hydrogen bonds between neighbouring groups, and hydrophobic interactions provide the necessary cohesion in the α -keratin arrangement [18–20]. The fact that ch-OSA supplementation partially prevents the loss in tensile strength suggests a structural effect of ch-OSA on hair fibers. An interaction with keratin is possible considering that orthosilicic acid (Si(OH)₄) is the prevalent chemical form of silicon in physiological fluids, and that silanol groups in orthosilicic acid are known to form complexes with amino acids [14] and peptides [15].

The cross-sectional area increased significantly after 9 months supplementation with ch-OSA compared to baseline, whereas in the placebo group no change was observed. Again this suggests that ch-OSA has a structural influence on keratin fibers or on the hair follicle. In a previous study, it was demonstrated that collagen type I synthesis was stimulated in skin fibroblasts by physiological concentrations of orthosilicic acid. Since the hair follicle is embedded in a collagen rich matrix, stimulation of collagen synthesis might influence the flow of nutrients to the hair follicle resulting in an effect on keratin formation. While most of the hair structure arises from epidermal keratinocytes, a specialized population of fibroblasts called the dermal papilla [6] controls hair growth [23]. The dermal papilla are located at the base of the hair shaft. Elliott et al. [17] report that the volume of the dermal papilla, including its surrounding dermal matrix, determines the volume of the hair follicle. This may provide a mechanism to explain the increase in cross-sectional area seen in the treatment group. If ch-OSA supplementation increased collagen synthesis by the fibroblasts of the dermal papilla, the result might be an increase in the volume of the dermal papilla and consequently the cross-sectional area of the hair shaft.

The cross-sectional area correlated strongly with the elastic gradient and the break load but not with break stress. Such a correlation was not found in other studies [33]. Interestingly, the change in Si (baseline versus 9 months) excretion was positively correlated with the change in cross-sectional area. Considering that urinary excretion is

the major route of silicon excretion in man, this correlation suggests that hair morphology is influenced by intake of bioavailable silicon.

To our knowledge, the present study is the first randomized, double blind placebo-controlled trial that illustrates the positive effect of an oral mineral supplement on hair morphology and tensile strength. Supplementation of choline-stabilized orthosilicic acid helps to reduce loss in hair elasticity and strength and improves hair thickness.

Acknowledgments ch-OSA was developed by Dirk Vanden Berghe for Bio Minerals n.v. This study was supported by a grant of Bio Minerals n.v. The Institute Dr. Schrader (Holzminden, Germany) was contracted as an independent research organization for the recruitment of volunteers and for the analysis of hair parameters.

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