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Clinical Study

A Clinical Trial to Investigate the Effect of Cynatine HNS on Hair and Nail Parameters

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Objective. A new, novel product, Cynatine HNS, was evaluated for its effects as a supplement for improving various aspects of hair and nails in a randomized, double-blind, placebo-controlled clinical trial. *Methods*. A total of 50 females were included and randomized into two groups. The active group ($n = 25$) received 2 capsules containing Cynatine HNS, comprised of Cynatine brand keratin (500 mg) plus vitamins and minerals, per day, and the placebo group ($n = 25$) received 2 identical capsules of maltodextrin per day for 90 days. End points for hair loss, hair growth, hair strength, amino acid composition, and hair luster were measured. End points were also measured for nail strength and the appearance of nails. *Results*. The results show that subjects taking Cynatine HNS showed statistically significant improvements in their hair and nails when compared to placebo. *Conclusion*. Cynatine HNS is an effective supplement for improving hair and nails in 90 days or less. EudraCT number is 2014-002645-22.

1. Introduction

In recent years, the dietary supplement use has increased both in Europe and in the USA with many physicians recommending their use [1, 2]. A survey of health professionals conducted in 2008 found that 66% of dermatologists ($n = 300$) recommended dietary supplements to patients in relation to skin, hair, and nail health and 79% of them personally used supplements [1]. The use of bioactive ingredients at concentrated doses found in dietary supplements can efficiently modulate the physiological processes better than the single ingredients in foods since heat or mechanical treatment of food before eating can enhance or reduce its bioavailability or activity [3, 4]. The benefits of food constituents may therefore differ if the same bioactive substances are present in nutraceutical formulations. In the case of nails and hairs the classical route of treatment is the use of topical application as well as shampoos. Nowadays, another means to improve nails and hairs is through oral administration (food and dietary supplements). The advantage of the oral administration route is that blood delivers nutraceutical bioactive compounds continuously to all compartments of hairs and nails.

Different studies on dietary supplements are arising in the scientific literature confirming the efficacy of dietary supplementation on maintaining and improving skin, hairs, and nails conditions. In 2007, Jacquet et al. [3] reported the efficacy of a dietary supplement containing 100 mg Shark Cartilage, 1.6 mg vitamin B_2, 6 mg vitamin B_5, 2 mg vitamin B_6, 0.150 mg vitamin B_8, and 350 mg fish oil (omega 3 PUFA) on skin, hairs, and nails in two open clinical trials (total of 52 women). During 58 days of this trial the product caused improvement in skin hydration, decrease of wrinkle depth/volume, a significant decrease of hair loss, and an improvement of nail conditions. Authors concluded that the product was effective in improving many signs of aging, such as skin appearance, nails, and hair. Other studies demonstrate the efficacy of oral minerals (i.e., zinc and iron) [5–9], B-vitamins [7, 10, 11], and L-cystine [7, 12] on hairs and nails. Some of these studies demonstrate that oral supplementation can have a positive effect on hairs or nails while some others

demonstrate that the lack of nutrient intakes with the diet has a detrimental role on hairs and nail conditions. However, some studies lack the dose-relationship effect, employed methods are not reliable or standardized, and the study design sometimes does not take the placebo group into account.

Cynatine HNS contains a protein called keratin, in a peptide form obtained by proprietary processing of New Zealand sheep wool. This novel ingredient is stable over a wide range of pH and under conditions of elevated temperature. Keratin protein is one of nature's richest sources of cysteine. Based on this, we hypothesized that Cynatine HNS may act synergistically with the cells' own antioxidant defense, boosting glutathione and other sulfur rich proteins and peptides. Keratin is the protein from which the majority of hair and nails are made. *In vitro* studies have shown that Cynatine HNS is highly bioavailable making it capable of delivering keratin peptides to the body, particularly to the hair and nails. Based on this, a randomized, double-blind, placebo-controlled study was conducted to examine the ability of Cynatine HNS to improve end points for hair loss, hair growth, hair strength, amino acid composition, and the appearance of hair on the head as well as the strength and appearance of nails.

2. Material and Methods

This study was a single-center, randomized, parallel group, double-blind, placebo-controlled 90-day intervention study in 50 subjects with signs of damaged hair and nails conducted at a single site in Italy (Farcoderm, University of Pavia). This clinical research study was done in accordance with the ethical principles for medical research involving human subjects (Helsinki Declaration, revised in 1983) and was approved by the internal ethical committee (Rif. 1032-11-SB). Informed consent documents were signed by all participants after study details were explained and each participant was evaluated for inclusion and exclusion criteria evaluated by dermatologists in the screening phase. Inclusion criteria for the study included being female and being between 40 and 71 years old, Caucasian, clinical signs of stressed or damaged hair, and an agreement not to use other possible cosmetic treatments which could interfere with the study. Exclusion criteria included subjects who did not fit the inclusion criteria, pregnant or breastfeeding women, use of a similar product to the active, and metabolism disorders. Once the inclusion criteria were met and consent forms were received, a screening number was assigned and entered into a screening and enrollment log. A randomization number was then given to each subject and a nonblinded employee provided the blinded examiner with the correct product at the beginning of each treatment period.

Once subjects were enrolled in the study they were provided with a base shampoo and conditioner to standardize the cosmetic habits for evaluation of product effects on hair as well as instructions. They were asked to use the base shampoo for five days and to return for their baseline visit (Day 0). At baseline, the dermatologist rechecked compliance of subjects to the protocol, evaluated baseline value for endpoints to be measured, hair (pull test, anagen/telogen evaluation, amino acid composition, mechanical properties, and appearance) and nails (clinical evaluation for nail status and breakage tendency), and supplied subjects with either active or placebo capsules and other information needed. A daily diary was also maintained in order to evaluate the habits of the volunteers in regard to foods and drinks during the first two weeks of the study as well as tobacco habits. At the end of the study period a questionnaire was filled out regarding the participants' personal opinion about the treatment (tolerability, acceptability, and efficacy). Subjects were asked to return to have the same endpoints for hair and nails measured at 30, 60, and 90 days.

The investigational product Cynatine HNS and placebo, provided by Roxlor Global, LLC, were given to the subjects as capsules packaged in blister packages. All Cynatine HNS capsules contained 250 mg Cynatine (keratin), 7.5 mg zinc, 9.0 mg vitamin B_3, 0.825 mg copper, 6.84 mg vitamin B_5, 1.0 mg vitamin B_6, and 0.150 mg vitamin B_8 (Biotin) on an active dose basis. Each placebo capsule, identical in size, shape, and color, contained the inactive ingredients maltodextrin 370 mg and magnesium stearate 5.0 mg. On Day 1, subjects were instructed to take two capsules daily in the morning after breakfast.

All subjects known to have started treatment and who returned to the clinic for at least one follow-up visit were included in the analyses. The Cynatine HNS group had one withdrawal after Day 30 giving an N value of 25 for Day 30 and 24 for Days 60 and 90. The placebo group had one withdrawal after Day 60 giving an N value of 25 for Days 30 and 60 and 24 for Day 90. Intragroup comparisons were made using Student's t-test and intergroup values were determined using Mann Whitney U Test.

The effects of both the active and placebo groups were measured on hair using five separate tests. These tests were a pull test, an anagen/telogen evaluation of the hair, the amino acid composition of the hair, the tensile strength of the hair, and the clinically evaluated appearance of the hair.

The pull test helps evaluate diffuse scalp hair loss. Gentle traction was exerted on a bunch of hairs (about 60) in three areas of the scalp (frontal, temporal, and occipital) and the number of extracted hairs was counted. The dermatologist takes a few strands between his/her thumb and forefinger and pulls them gently. In anagen phase, growing hair should remain rooted in place while hair in the telogen phase should come out easily. If the number of lost hairs is greater than 9, pull test is positive and suggestive of telogen effluvium. The subjects were asked to refrain from washing their hair 2-3 days before the pull test.

Anagen/telogen testing is performed by choosing a targeted area (mid-vertex) of approximately 1.8 cm^2 for clipping hair, which was dyed for gray and fair colored hair. Close-up digital photographs were immediately taken after shaving and 2 days later. The two photographs were compared by software that was able to determine if hair was in anagen phase (growing) or telogen phase (not growing). For the amino acid composition of the hair, hair samples were hydrolyzed in 6 M HCl aqueous solution. Amino acids were then separated by reverse-phase liquid chromatography and identified in an X-LC fluorimeter (model 3020FP). The amino acids measured

for this test are serine, glutamic acid, cystine, and methionine. The breakage force of a single hair fiber was evaluated by a dynamometer (Tensolab 2512A, Mesdan Lab). An average of 10 readings is reported.

The hair appearance is evaluated by a licensed dermatologist who assigns a value of one to three based upon the subject's hair brightness and luster. A score of 1 is dull and devoid of brightness, a score of 2 is basically dull and not so bright, and a score of 3 is shiny and bright.

The nail appearance and tendency to break were evaluated by a licensed dermatologist. The appearance of the nails is recorded in 5 either/or categories. These categories are Hard/Soft, Resistant/Fragile, Broken/Not Broken, Rough/Smooth, and Yellowish/White. The nails tendency to break is evaluated on Day 0 with a score of one to three. A score of 1 indicates that nails are flaked, are broken, or have a tendency to break, a score of 2 means nails are moderately flaked, broken, and a score of 3 indicates that neither nails are flaked, broken nor do they have a tendency to break. At Days 30, 60, and 90, the nails tendency to break is measured via a four-point scale. A score of 1 is no improvement, 2 is slight improvement, 3 is moderate improvement, and 4 is remarkable improvement. For the nail tendency to break measures, a subject with a score of 3 initially is not included in the analysis as there is no room for improvement. Eight subjects on Cynatine HNS had scores of 3, which lowers the respective N values by eight for each measure. Placebo had 10 subjects with initial scores of 3, lowering the respective N values by 10 for this calculation.

To evaluate the fact that the statistical analysis was accurate and reliable and that the sample size was large enough to detect variation of the measured parameter a *post hoc* power analysis was performed. The output of the power analysis clearly indicated that the sample sizes were large enough (power of at least 80%) to detect the differences obtained before and after treatment.

3. Results

3.1. Hair Measurement Results

Hair Pull Test. The subjects in the placebo group showed no change in number of hairs lost during the study time points 30 and 60 days. However, at the end of the study period there was a significant improvement compared to baseline ($P < 0.01$). Subjects on Cynatine HNS showed a statistically significant improvement in reducing hair loss throughout the test period. A statistically significant improvement was already seen within the Cynatine HNS group at Day 30 ($P < 0.001$) with a 16.9% improvement. This further improved within the Cynatine HNS group at Day 60 (38.9%, $P < 0.001$) and Day 90 (46.6%, $P < 0.001$). The Cynatine HNS group was trending towards significance at Day 30 ($P = 0.07$) and was statistically significant at Days 60 and 90 ($P < 0.001$ for both) when compared to placebo. Overall, Cynatine HNS showed a 12.5% reduction in hair loss over placebo at Day 30 and a 34.5% and 34.4% reduction at Days 60 and 90, respectively. Figure 1 shows the results for both groups over the 90-day time period.

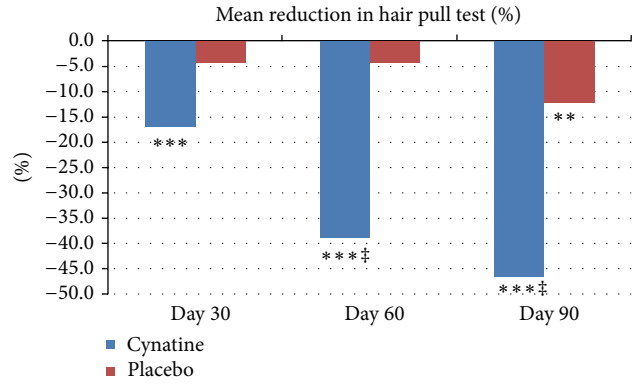

Figure 1: Mean percent reduction in hair pull test from baseline for placebo and Cynatine HNS. $^{**}P < 0.01$ and $^{***}P < 0.001$ within group to baseline; $^{‡}P < 0.001$ between groups to baseline.

Anagen/Telogen Test. The subjects in the placebo group showed no change in either anagen (growth phase) or telogen (nongrowth phase) phase of the hair cycle after 90 days. Subjects on Cynatine HNS showed statistically significant improvement in their anagen/telogen ratio. Both the telogen and anagen phases improved at Day 90 by 9.2% ($P < 0.001$) compared to baseline. This was also a statistically significant improvement compared to placebo at the end of the test period ($P < 0.001$).

Amino Acid Profile. Subjects taking placebo showed no improvement in the amino acid ratio of serine, glutamic acid, cystine, and methionine. At the end of the test period at 90 days the subjects on the active Cynatine HNS treatment showed a statistically significant increase in all 4 amino acids based on their ratio to total protein content. At Day 90, the mean percent increase of serine was 3.2% ($P < 0.001$), glutamic acid 3.5% ($P < 0.001$), cystine 8.6% ($P < 0.001$), and methionine 4.8% ($P < 0.001$) compared to baseline. At Day 90 these concentrations were all significantly different to placebo ($P < 0.001$). The increase of the amino acid ratio, especially of cystine which is a main component of Cynatine, also shows the bioavailability of Cynatine in the body. Figure 2 shows the results for both groups at baseline and Day 90.

Hair Tensile Strength. Subjects on placebo showed no statistical improvement in their hair strength at the end of the test period. The active group treated with Cynatine HNS showed a 5.9% improvement in hair strength at Day 90 ($P < 0.001$) compared to baseline as well as a statistically significant percent change to placebo at the end of the test period ($P < 0.001$).

Hair Appearance. Both groups in this test started with a mean score of 1.70 ± 0.5. Subjects on placebo showed no improvement in the mean score at Day 30 and an increase of 0.30 ($P < 0.01$) at Day 60 and no further improvement at Day 90. Subjects who were treated with Cynatine HNS showed a statistically significant improvement at all times measured compared to both baseline and placebo. At Day 30 the mean increase in appearance scores was 0.30 ($P < 0.01$),

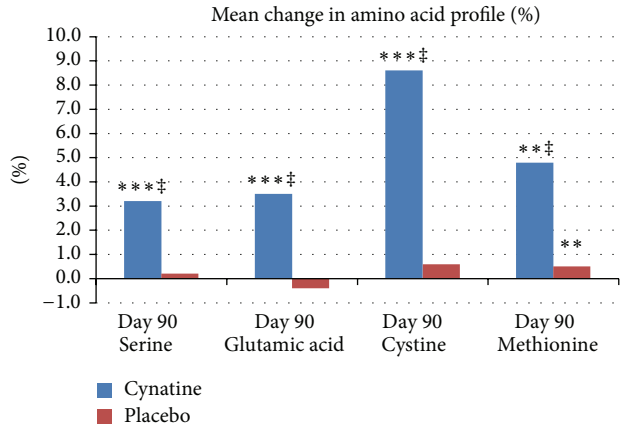

Figure 2: Mean percent change in amino acid profile from baseline for placebo and Cynatine HNS. $^{**}P < 0.01$ and $^{***}P < 0.001$ within group to baseline; $^{‡}P < 0.001$ between groups to baseline.

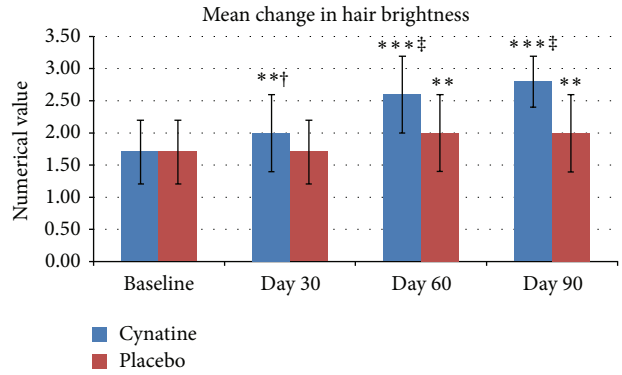

Figure 3: Mean change in hair brightness from baseline for placebo and Cynatine HNS. $^{**}P < 0.01$ and $^{***}P < 0.001$ within group to baseline; $^{†}P < 0.05$, $^{‡}P < 0.001$ between groups to baseline.

at Day 60 it was 0.90 ($P < 0.001$), and at Day 90 it was 1.10 ($P < 0.001$) when compared to baseline. The results at all time points are also statistically significant to placebo (Day 30 $P < 0.05$, Day 60 $P < 0.001$, and Day 90 $P < 0.001$). The percent improvement compared to placebo was 17.6% at Day 30, 35.3% at Day 60, and 47.1% at Day 90. It also should be noted that 23 out of 24 subjects showed an improvement with Cynatine HNS, while only 8 of 24 showed any improvement on placebo. The results at all times points for both groups are shown in Figure 3.

3.2. Nail Measurement Results

Nails Tendency to Break. Subjects on placebo showed no statistical improvement over the 90-day time frame. On the improvement scale used in this test placebo had a score of 1.00 at Day 30, showing no improvement, and a mean score of 1.30 at Day 90, showing very limited improvement. Subjects on Cynatine HNS have scores of 1.90 at Day 30, 2.20 at Day 60, and 2.7 at Day 90, showing slight to moderate improvement in nails according to the grading scale used in this test. At all three time points the results compared to placebo were statistically significant ($P < 0.001$). 87.5% of subjects taking Cynatine HNS showed an improvement in their nails tendency to break, whereas only 28.5% of subjects on placebo showed any improvement.

Appearance of Nails. In the Hard/Soft quality of nails, hard is the desirable trait. In the placebo group, at baseline 41.7% of the subjects had hard nails and at Day 90 58.3% had hard nails. In the Cynatine HNS group, at baseline 37.5% had hard nails and at Day 90 87.5% had hard nails. A resistant nail is the beneficial quality in the Resistant/Fragile measure. At baseline, the placebo group had 41.7% of its subjects with resistant nails and at Day 90 58.3% had resistant nails. In the Cynatine HNS group, 33.3% had resistant nails at baseline and 87.5% had resistant nails at Day 90. A not broken nail is the desired result in the Broken/Not Broken measure. At baseline, 50% of the placebo group had no broken nails and 58.3% had no broken nails at Day 90. At baseline, 54.3% of the Cynatine HNS group had no broken nails and 87.5% had no broken nails at Day 90. In the Rough/Smooth measure, smooth is the desired trait. At baseline, 66.7% of the placebo group had smooth nails and at Day 90 79.2% had smooth nails. At baseline, 62.5% of the Cynatine HNS group had smooth nails and by Day 60 100% of the subjects had smooth nails. A white or natural color is desired for the nail and at baseline 83.3% had this trait in the placebo group compared to 87.5% at Day 90. 79.2% of the Cynatine HNS group had white nails at baseline and by Day 60 100% of the subjects had white nails.

All five measures of the nails appearance in the Cynatine HNS group are statistically significant to both baseline and placebo by Day 60 and all have a value of $P < 0.02$ or less at Day 90. While being still statistically significant, the P values in the appearance measures are larger than other measures in the study because of the limited room for improvement in many of the measures especially when compared to placebo. However, when analyzing the number of people showing improvement where possible, the largest percentage increase for placebo in any measure is 16.7%. This equates to four total people showing improvement at most in any measure on placebo. The lowest final score in the Cynatine HNS group is 87.5% in three of the measures. In those three measures, only three people total in the active group did not achieve the desired result and in the other two measures 100% of the subjects achieved the desired result.

3.3. Adverse Events/Withdrawals. There were no adverse events reported during the study, with 2 withdrawals. Both withdrawals were deemed by the principle examiner not to be related to either the active or the placebo group. Both withdrawals were because the subject claimed intolerance to the product, but this occurred after 30 days in the active group and 60 days in the placebo group. Based on this the examiner determined that it was individual susceptibility that was the cause of the intolerance. Both the active and the placebo groups were well tolerated in study with 100% of the subjects finishing the study saying they were well tolerated. Subjects in the active group also gave the product either an excellent or a good score in the products acceptability. Based on this,

Cynatine HNS was found to be safe and well tolerated in this study.

4. Discussion and Conclusion

A eutrophic effect for hair on the head was seen after 3 months of treatment. This was demonstrated by the decrease of hair shedding in the pull test. The Cynatine HNS group showed significantly less hair loss after 30, 60, and 90 days which were significantly different to the placebo group. This could be explained by the improvement of the anagen and telogen phases of the hair. In the Cynatine HNS group both growth phase (anagen) and stationary phase (telogen) improved resulting in less hair being pulled out. This was not seen in the placebo group. Amino acid composition of serine, glutamic acid, cystine, and methionine improved in the Cynatine HNS group significantly to give the hair a better quality. This can be explained by the addition of the various bioavailable amino acids from keratin, which is part of the Cynatine HNS formula. With an improvement in the hair quality its mechanical properties also improved significantly at Day 90 compared to placebo. Even the clinical evaluation by the physician concluded that hair shininess and brightness had improved in the Cynatine HNS group in 87.5% of the subjects compared to only 16.7% in the placebo group. An overall assessment of hair brightness showed a 64.7% change compared to only 17.6% in placebo. This is more than a 3x improvement in hair brightness at the end of the test period.

Nails also improved their condition after 1, 2, and 3 months of treatment as demonstrated by the increase of the subjects having hard and resistant nails and the decrease of the subjects having broken and roughened nails. Hardness of nails improved from 37.5% of subjects reporting hard nails to 87.5% at the end of 90 days. That goes hand in hand with the improvement in resistance and none broken nails. The placebo group scored in all categories below 17%. As the nails improved in hardness and resistance they also improved significantly in smoothness at Day 90 compared to placebo. They also changed to a more normal color than the yellow discoloration seen. The clinical evaluation by a physician also went along the same lines and an improvement in tendency to break was seen in 87.5% of subjects as compared to only 28.6% in the placebo group.

In a questionnaire administered after completion of the study, participants were asked to rate how effective they felt the products were. Not surprisingly, the placebo scored poorly in the questionnaire as 87.5% of the participants felt that it was ineffective for hair and 84.4% felt that it was poor for nails. In the Cynatine HNS group, 91.7% of the participants felt that the product was sufficient for hair with 50% feeling that the product was either very good or excellent. For nails, 87.5% of the Cynatine HNS group felt that the product was sufficient with 66.6% finding it very good or excellent.

Based on the results of this study, it would be recommended that further clinical analysis should be performed on Cynatine HNS. In order to better analyze the effect of Cynatine HNS on hair and nails a study which includes men and women, a larger sample size, and a longer duration should be performed. Additionally it would be beneficial to look at a comparison of Cynatine with and without the additional vitamins and minerals.

In conclusion, the results obtained for the Cynatine HNS group were statistically different from that obtained for the placebo group, demonstrating that Cynatine HNS had a significant influence on the quality of skin, hair, and nails. Cynatine HNS contains ingredients that are all seen as nutrients for skin, hair, and nails. Keratin is a major structural component of the hair and nails which can be seen by the influence Cynatine HNS has on the quality of hair and nails.

Conflict of Interests

Robert H. Veghte is the General Manager of Roxlor Global, LLC. Neither Christina Beer nor Simon Wood is an employee of Roxlor Global, LLC, nor do they receive any royalty or payment based on performance of the product; they do however receive consulting fees on a per job basis from Roxlor Global, LLC.

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A randomized, double-blind, placebo-controlled clinical trial to investigate the effect of Cynatine® HNS on skin characteristics

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Keywords: Cynatine® HNS, healthy skin, reduced wrinkles, skin elasticity, skin moisture

Synopsis

OBJECTIVE: A new, novel product, Cynatine® HNS was evaluated for its effects as a supplement for improving various aspects of skin in a randomized, double-blind, placebo-controlled clinical trial.

METHODS: A total of 50 females were included and randomized into two groups. The active group (n = 25) received two capsules totalling of Cynatine® HNS, comprised of Cynatine® brand keratin (500 mg) plus vitamins and minerals, per day, and the placebo group (n = 25) received two identical capsules of maltodextrin per day for 90 days. End points for skin moisture, skin elasticity, wrinkle reduction, skin compactness and skin appearance were measured.

RESULTS: The results show that subjects taking Cynatine® HNS showed statistically significant improvements in their skin when compared with placebo.

CONCLUSION: Cynatine® HNS is an effective supplement for improving skin in 90 days or less.

Résumé

OBJECTIF: Un nouveau produit, Cynatine® HNS a été évalué pour ses effets comme un complément pour améliorer divers aspects de la peau dans un essai clinique en double randomisé, contrôlé contre placebo.

MÉTHODES: Un total de 50 femmes a été inclus et randomisé en deux groupes. Le groupe actif (n = 25) a reçu two capsules par jour totalisant 500 mg de Cynatine® HNS, composé de kératine de la marque Cynatine® ainsi que des vitamines et des minéraux, et le groupe placebo (n = 25) a reçu two capsules identiques de maltodextrine par jour pendant 90 jours.

Les paramètres humidité de la peau, élasticité de la peau, réduction des rides, la compacité de la peau et l'apparence de la peau ont été mesurées.

RÉSULTATS: Les résultats montrent que les sujets prenant Cynatine® HNS ont affiché des améliorations statistiquement significatives dans leur peau par rapport au placebo.

CONCLUSION: Cynatine® HNS est un complément efficace pour améliorer la peau en 90 jours ou moins.

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Introduction

In recent years, dietary supplement use has increased in Europe and the U.S.A. with many physicians recommending their use [1, 2]. A survey of health professionals conducted in 2008 found that 66% of dermatologists (n = 300) recommended dietary supplements to patients for skin, hair and nail health and 79% of them personally used supplements [1]. The use of bioactive ingredients at concentrated doses found in dietary supplements can efficiently modulate the physiological processes better then the single ingredients in foods, as heat or mechanical treatment of food before eating can enhance or reduce its bioavailability or activity [3, 4]. The benefits of food constituents may therefore differ if the same bioactive substances are present in nutraceutical formulations. In the case of skin, the classical route of treatment is topical application. Nowadays, another means to improve the skin is through oral administration (food and dietary supplements). The advantage of the oral administration route is that blood delivers nutraceutical bioactive compounds continuously to all skin compartments (i.e. epidermis, dermis, hairs and nails).

Different studies on dietary supplements are arising in the scientific literature confirming the efficacy of dietary supplementation on maintaining and improving skin conditions. In 2007, Jacquet *et al.* [3] reported the efficacy of a dietary supplement containing 100 mg Shark cartilage, 1.6 mg vitamin B_2, 6 mg Vitamin B_5, 2 mg Vitamin B_6, 0.150 mg Vitamin B_8 and 350 mg Fish oil (Omega 3 PUFA) on skin, hairs and nails in two open clinical trials (total of 52 women). During 58 days of this trial, the product caused improvement in skin hydration, decrease in wrinkle depth/volume, a significant decrease in hair loss and an improvement in nail conditions. Authors concluded that the product was effective in improving many signs of ageing, such as skin appearance, nails and hair. Others studies demonstrate the efficacy of oral antioxidants (i.e. carotenoids and polyphenols) [4–7] and peptides [8, 9] on skin. Some of these studies demonstrate that oral supplementation can have a positive effect on skin, whereas some other demonstrate that the lack of nutrient intakes with the diet has a detrimental role on skin conditions. However, some studies lack the dose-relationship effect, employed methods are not reliable or standardized, and the study design sometimes does not take the placebo group into account.

Cynatine® HNS contains a protein called keratin, in a peptide form obtained by proprietary processing of New Zealand sheep

wool. Keratin protein is one of nature's richest sources of Cysteine. We thus hypothesized Cynatine® HNS may act synergistically with the cells' own antioxidant defence, boosting glutathione and other sulphur-rich proteins and peptides. Keratin is found in many different layers of the skin. Outer skin cells are filled with keratin, helping the skin to retain moisture and delivering the skin's elasticity and smoothness. The outer keratin layer also protects the underlying layers of skin. *In vitro* studies have shown that Cynatine® HNS is highly bioavailable, making it capable of delivering keratin peptides to the body, particularly to the skin. Based on this, a randomized, double-blind, placebo-controlled study was conducted to examine the ability of Cynatine® HNS to improve end points for the health and appearance of facial skin.

Material and methods

This study was a single-centre, randomized, parallel group, double-blind, placebo-controlled 90-day intervention study in 50 subjects with signs of skin ageing conducted at a single site in Italy (Farcoderm, University of Pavia). This clinical research study was carried out in accordance with the Ethical Principles for Medical Research involving Human Subjects (Helsinki Declaration, revised in 1983) and was approved by the internal ethical committee (Rif. 1032-11-SB). Informed consent documents were signed by all participants after study details were explained, and each participant was evaluated for inclusion and exclusion criteria evaluated by dermatologists in the screening phase. Inclusion criteria for the study included being female and being between 40 and 71 years old, Caucasian, signs of chrono or photo ageing and an agreement not to use other possible cosmetic treatments which could interfere with the study. Exclusion criteria included subjects who did not fit the inclusion criteria, pregnant or breastfeeding women, use of a similar product to the active, skin condition or disease, use of tanning beds or skin rejuvenation procedures in prior six months, dermatological treatments and metabolism disorders. Once the inclusion criteria were met and consent forms were received, a screening number was assigned and entered into a Screening and Enrolment Log. A randomization number was then given to each subject, and a non-blinded employee provided the blinded examiner with the correct product at the beginning of each treatment period.

Once subjects were enrolled in the study, they were provided with a base skin cream to standardize the cosmetic habits for evaluation of product effects on skin as well as instructions. They were asked to use the base cream for five days, as well as not to apply any cosmetics or make-up to the face for the five days, and to return for their baseline visit (Day 0). At baseline, the dermatologist rechecked compliance of subjects to the protocol, evaluated baseline value for endpoints to be measured: Skin (moisture, elasticity, wrinkles, protein content and skin appearance) and supplied subjects with either active or placebo capsules and other information needed. A daily diary was also maintained to evaluate the habits of the volunteers in regard to foods and drinks during the first two weeks of the study as well as tobacco habits. At the end of the study period, a questionnaire was filled out regarding the participants' personal opinion about the treatment (tolerability, acceptability and efficacy). Subjects were asked to return to have the same endpoints for skin measured at 30, 60 and 90 days.

The investigational product Cynatine® HNS and placebo, provided by Roxlor Global, LLC, were given to the subjects as capsules packaged in blister packages. Each Cynatine® HNS capsules contained 250 mg Cynatine® (keratin), 7.5 mg Zinc, 9.0 mg Vitamin B_3, 0.825 mg Copper, 6.84 mg Vitamin B_5, 1.0 mg Vitamin B_6 and 0.150 mg Vitamin B_8 (Biotin) on an active dose basis. Each placebo capsule, identical in size, shape and colour, contained the inactive ingredients maltodextrin 370 mg and magnesium stearate 5.0 mg. On Day 1, subjects were instructed to take two capsules daily in the morning after breakfast.

All subjects known to have started treatment and who returned to the clinic for at least one follow-up visit were included in the analyses. The Cynatine® HNS group had one withdrawal after Day 30 giving a N value of 25 for Day 30 and 24 for Days 60 and 90. The placebo group had one withdrawal after Day 60 giving an N value of 25 for Days 30 and 60 and 24 for Day 90. Intragroup group comparisons were made using Student's t-test, and intergroup values were determined using Student's *t*-test.

The effects of both the active and placebo were measured on skin using five separate tests. These tests were a skin moisture, skin elasticity, three measures of wrinkles, protein content and skin appearance.

The skin moisture measurement was based on the internationally recognized Corneometer® method (Courage + Khazaka, electronic GmbH), which is based on the dielectric constant of water. The probe shows capacity changes in accordance to moisture content of the skin. An electric scatter field penetrates the first layer of skin and determines its dielectricity. Neither a galvanic reaction between the device and the skin nor a polarization effect exists unlike impedance measurements.

The skin elasticity method was based on suction/elongation and the successive release of the skin inside a probe. This method generates negative pressure which is able to draw the skin into the probe. The release phase, where pressure is released back to zero, the skin returns back to its resting stage. A Cutometer® MPA 580 (Courage + Khazaka, electronic GmbH) was used to evaluate the depth reached by the skin inside the probe during the suction and release phases. The visco-elastic properties of the skin can then be calculated. The ratio between maximum skin elongation (Uf) and deformation (Ua) was used to reach the results. This parameter is known as R2 and indicates the skins ability to return to its baseline stage after deformation stress.

Skin Wrinkle measures were quantitatively assessed using Primos 3D Analysis (GF Messtechnik GmbH). This method is a non-contact *in vivo* skin instrument based on structured light projection. In conjunction with comprehensive 3D measurement and evaluation software, the sensor allows the evaluation of wrinkle depth, volume and roughness. This method calculates the Ra parameter (ISO 4287, DIN 4768), Rz parameter (ISO 4287, DIN 4768) and wrinkle depth.

The protein content (cohesivity) is measured using the Corneofix® foil (Courage + Khazaka, electronic GmbH) method. Non-invasive samples of 10 layers of the *stratum corneum* from a clean face were obtained to determine the protein content. Evaluating skin cohesivity in terms of its protein content is useful to assess compacting efficacy of the treatment, which implies a reduction in the amount of protein remaining on the foil. The Lowry method is used to measure the protein content. It is based on the ability of copper to bind to proteins under alkaline conditions, and when Folin phenol reagent is added, a complex is formed with the protein that can be seen at 550 nm.

Skin appearance was evaluated by a registered dermatologist at baseline and is given an initial score in two categories, skin wrinkles and skin compactness. The subjects then return and were evaluated at Days 30, 60 and 90 and given an improvement score of one

Table I Clinical classification of skin wrinkles and compactness at baseline

Classification of skin wrinkles at T0	Score
No wrinkle. No visible wrinkle, continuous skin line	0
Very shallow yet visible wrinkle	0.5
Fine wrinkle. Visible wrinkle and slight indentation	1
Visible wrinkle and clear indentation	1.5
Moderate wrinkle. Clearly visible wrinkle	2
Prominent and visible wrinkle	2.5
Deep wrinkle. Deep and furrow wrinkle	3
Clinical classification of skin compactness at T0	
Not compact/tonic skin (Low elasticity)	1
Insufficient compact/tonic skin (low elasticity)	2
Compact/tonic skin (elastic skin)	3
Well compact/tonic skin (very elastic skin)	4

through four. A score of one shows no variation, two shows slight improvement, three shows moderate improvement and four shows remarkable improvement. The base scores are listed in Table I.

To evaluate that the statistical analysis was accurate and reliable and that the sample size was large enough to detect variation of the measured parameter, a *post hoc* power analysis was performed. The output of the power analysis clearly indicated that the sample sizes were large enough (power of at least 80%) to detect the differences obtained before and after treatment.

Results

Skin measure results

Skin moisture

This clinical trial was performed in winter, when it would be expected that a person's skin would lose moisture based on the colder, drier days in the testing area and a propensity to stay indoors exposing the skin to heat. This can be seen in the placebo results, where decreases of 7.9% at Day 30 ($P < 0.01$), 12.9% at Day 60 ($P < 0.001$) and 19.3% at Day 90 ($P < 0.001$) were seen. The group on the active product, Cynatine® HNS, saw statistically significant increases of 5.4% at Day 30 ($P < 0.05$), 9.3% at Day 60 ($P < 0.05$) and 11.1% at Day 90 ($P < 0.001$). When compared with placebo, the active group showed an overall improvement of 13.3% at Day 30, 22.2% at Day 60 and 30.4 at Day 90. All three time points were statistically significant intergroup ($P < 0.01$ Day 30, $P < 0.001$ Days 60 and 90). Furthermore, almost 80% of the subjects taking Cynatine® HNS showed an increase in skin moisture, whereas only 8% on placebo showed an increase. The mean percent change in skin moisture for all time periods can be seen in Fig. 1.

Skin elasticity

Subjects taking placebo showed a decrease in their skin elasticity at all time points compared with Baseline. At Days 30 and 60, there was a 2.3% and 3.2% loss of elasticity ($P < 0.05$), respectively, and at Day 90, there was a 4.6% ($P < 0.01$) loss. At all time points, the group on Cynatine® HNS showed an increase in skin elasticity. At Day 30, there was a 4.3% increase ($P < 0.05$), at Day 60, there was a 7.2% increase ($P < 0.01$), and at Day 90, there was a 12.2% increase ($P < 0.001$). Overall, there was a 16.8% improvement over placebo at Day 90 for skin elasticity. At Day 60 ($P < 0.01$) and Day 90 ($P < 0.001$), the results of the active group were statistically significant to placebo. 87.5% of subjects in the active group also showed an increase in skin elasticity during the study. Mean per cent changes in skin elasticity for all time periods can be seen in Fig. 2.

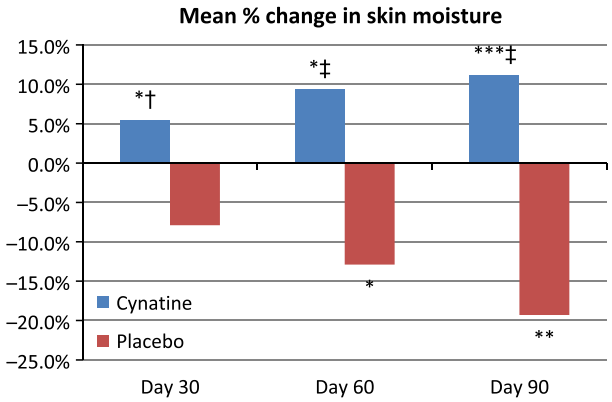

Figure 1 Mean% change in skin moisture for Cynatine® HNS and Placebo. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ within group to baseline, †$P < 0.01$, ‡$P < 0.001$ between groups to baseline.

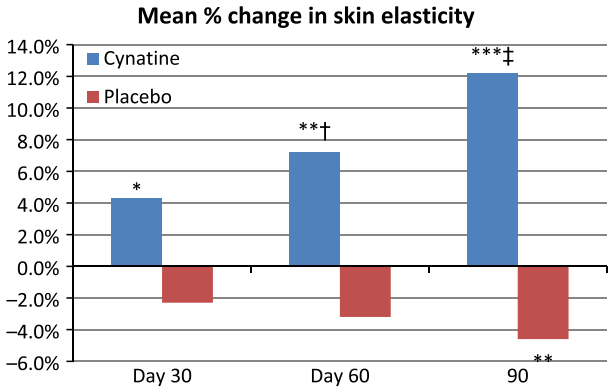

Figure 2 Mean% change in skin elasticity for Cynatine® HNS and Placebo. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ within group to baseline, †$P < 0.01$, ‡$P < 0.001$ between groups to baseline.

Skin wrinkles

Skin wrinkles were measured using three separate parameters. With the Ra parameter, the Ra increases as roughness increases. The group on placebo did not show statistically significant changes at Days 30 and 60, but did show a 7.8% increase in Ra at Day 90 ($P < 0.01$). The group on Cynatine® HNS showed statistically significant decrease to baseline at all three time points, with a 4.7% decrease at Day 30 ($P < 0.05$), a 7.3% decrease at Day 60 ($P < 0.001$) and a 10.1% decrease at Day 90 ($P < 0.001$). The intergroup results were also statistically significant at Day 60 ($P < 0.01$) and Day 90 ($P < 0.001$). With the Rz parameter, as Rz increases, roughness increases. The placebo group did not show any statistically significant results for any time period. The active group showed statistically significant different results to baseline at all time points including a 2.6%

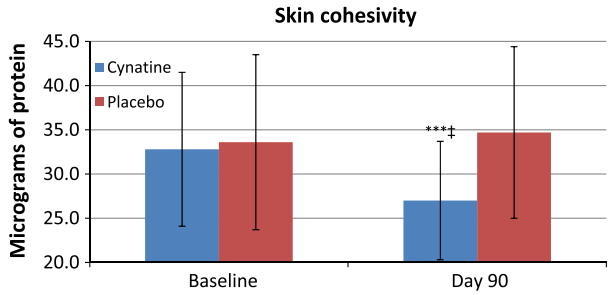

Figure 3 Mean change in skin protein content for Cynatine® HNS and Placebo. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ within group to baseline, †$P < 0.01$, ‡$P < 0.001$ between groups to baseline.

reduction at Day 30 ($P < 0.05$), a 6.5% reduction at Day 60 ($P < 0.001$) and an 8.6% reduction at Day 90 ($P < 0.001$). The Cynatine® HNS group was statistically significant different to placebo at Days 60 and 90 (both $P < 0.001$). The placebo showed no statistically significant results in changes in wrinkle depth at any time period. The Cynatine® HNS group showed statistically significant different results to baseline at all time points, with a reduction of 11.5% at Day 90 ($P < 0.001$). The active results were statistically significant different to the placebo at Days 60 and 90 ($P < 0.001$).

Protein content

The subjects in the placebo group showed no improvement in skin protein content from baseline to Day 90. Subjects in the Cynatine® HNS group improved their skin protein content significantly to baseline at Day 90 based on their being less protein caught in the Corneoflex foils (15.9% less protein caught; $P < 0.001$). The Cynatine® HNS group also significantly improved in skin protein content compared with placebo at Day 90 with a difference of 19.7%. over placebo ($P < 0.001$). 95.8% of the subjects in the active group showed an improvement in skin cohesivity. The mean change in skin protein content can be seen in Fig. 3.

Skin appearance

In the dermatologist evaluation of skin wrinkles, no subjects in the placebo group showed any clinical improvement in skin wrinkledness at Days 30 and 60. Only 1 subject in the placebo group improved at Day 90. In the Cynatine® HNS group 5 (20.8%), 13 (54.2%) and 14 (58.3%) subjects improved at 30, 60 and 90 days, respectively. These improvements were all statistically significant ($P < 0.05$) compared with the placebo group. Dermatologist evaluation for skin compactness showed only 1 subject at Day 30 and two subjects each at Days 60 and 90 in the placebo group showed any clinical improvement in compactness. In the Cynatine® HNS group 6 (25%), 9 (37.5%) and 12 (50%) subjects improved at 30, 60 and 90 days, respectively. These improvements were all statistically significant ($P < 0.01$; $P < 0.05$; $P < 0.01$, respectively) to the placebo group.

Adverse events and withdraws

There were no adverse events reported during the study, with two withdrawals. Both withdraws were deemed by the principle examiner not to be related to either the active or placebo. Both withdraws were because the subject claim intolerance to the product, but this occurred after 30 days in the active and 60 days in the placebo. Based on this, the examiner determined that it was individual susceptibility that was the cause of the intolerance. Both the active and placebo were well tolerated in study with 100% of the subjects finishing the study saying that they were well tolerated. Subjects in the active group also gave the product either an excellent or good score in the products acceptability. Based on this, Cynatine® HNS was found to be safe and well tolerated in this study.

Discussion and conclusion

Study results clearly demonstrated a positive effect of Cynatine® HNS on maintaining normal facial skin. An improvement in skin trophism starting from the second month of Cynatine® HNS supplementation was seen in almost all skin parameters tested. Skin moisture improved in the Cynatine® HNS group 30 days from the use of the ingredient, and this went hand in hand with an improvement in elasticity as well when compared with placebo. The placebo group showed no improvement in elasticity but rather decreased both in moisture and elasticity parameters or stayed the same compared with baseline. As the roughness of the skin decreased in the Cynatine® HNS group, the smoothness increased significantly at Days 60 and 90 compared with placebo. No changes were seen in the placebo group. All skin surface properties improved in the Cynatine® HNS group from Day 60 including the measured wrinkle depth, which had significantly improved (reduced) compared with baseline and placebo. The same conclusion was determined in the clinical assessment by the physician with a total of 54.2% and 58.3% improvement compared with placebo. This was a 13× larger improvement in the Cynatine® HNS group at Day 90. Clinical evaluation of skin compactness also saw a 50% improvement in the Cynatine® HNS group as compared to placebo (8.3%). The variation in all the parameters investigated is indicative that Cynatine® HNS is efficacious in decreasing the effects of the photo or chrono-ageing on skin.

The results obtained for the Cynatine® HNS group were statistically different from those obtained for the placebo group, demonstrating that Cynatine® HNS had a significant influence on the quality of skin. Cynatine® HNS contains ingredients that are all seen as nutrients for skin. Keratin is a major structural block of the human outer layer and thus plays a significant role in the quality of the skin. It provides all the essential and non-essential amino acids for proper skin protein formation [10, 11]. The vitamins all play a vital role in the essential pathway to produce healthy skin and retain moisture as well [12]. Supplementing with Cynatine® HNS can improve skin characteristics because of chrono or photo ageing. It is an easy alternative to using lotions and crèmes and supports the skin from the inside out.

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Conflict of Interest: Robert H. Veghte is the General Manager of Roxlor. Neither Christina Beer nor Simon Wood is an employee of Roxlor nor do they receive any royalty or payment based on performance of the product. They do, however, receive consulting fees on a per-job basis from Roxlor.

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