Pycnogenol® Effects on Skin Elasticity and Hydration Coincide with Increased Gene Expressions of Collagen Type I and Hyaluronic Acid Synthase in Women

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Key Words
Pycnogenol® · Pine bark extract · Skin hydration · Skin elasticity · Collagen expression · Hyaluronic acid synthase-1

Abstract

Introduction and Objectives: In recent years there has been an increasing interest in the use of nutritional supplements to benefit human skin. Molecular evidence substantiating such effects, however, is scarce. In the present study we investigated whether nutritional supplementation of women with the standardized pine bark extract Pycnogenol® will improve their cosmetic appearance and relate these effects to expression of corresponding molecular markers of their skin. Materials and Methods: For this purpose 20 healthy postmenopausal women were supplemented with Pycnogenol for 12 weeks. Before, during and after supplementation, their skin condition was assessed (i) by employing non-invasive, biophysical methods including corneometry, cutometry, visioscan and ultrasound analyses and (ii) by taking biopsies and subsequent PCR for gene expression analyses related to extracellular matrix homeostasis. Results: Pycnogenol supplementation was well tolerated in all volunteers. Pycnogenol significantly improved hydration and elasticity of skin. These effects were most pronounced in women presenting with dry skin conditions prior to the start of supplementation. The skin-physiological improvement was accompanied by a significant increase in the mRNA expression of hyaluronic acid synthase-1 (HAS-1), an enzyme critically involved in the synthesis of hyaluronic acid, and a noticeable increase in gene expression involved in collagen de novo synthesis. Conclusions: This study provides skin-physiological and for the first time molecular evidence that Pycnogenol supplementation benefits human skin by increasing skin hydration and skin elasticity. These effects are most likely due to an increased synthesis of extracellular matrix molecules such as hyaluronic acid and possibly collagen. Pycnogenol supplementation may thus be useful to counteract the clinical signs of skin aging.
aimed at defying skin aging. The vast majority of these claims are based either on in vitro studies with no or limited in vivo relevance or on in vivo studies in the absence of relevant molecular investigations. Skin aging, however, is accompanied and orchestrated by a number of very well-characterized molecular changes which are of established physiological relevance and, therefore, well-known targets for many effective cosmetic anti-aging strategies [1, 2]. It is currently not known whether nutritional supplements which are suggested to exert anti-skin aging effects can indeed exert beneficial effects in human skin at the molecular level. Demonstration of such effects, however, would provide a scientific rationale for any claimed skin anti-aging effects.

In the present human in vivo study, we have addressed this question by focusing on the nutritional supplement Pycnogenol®. Pycnogenol represents a standardized bark extract from the French maritime pine (Pinus pinaster Ait) in compliance with US pharmacopoeial requirements. We have chosen this product because the extract is standardized to contain 70±5% procyanidins, oligomers of catechin and epicatechin subunits, taxifolin and a range of phenolic acids, derivatives of benzoic and cinnamic acids. In other words, Pycnogenol contains a variety of bio-active molecules which are known to exert beneficial effects on skin cells in vitro or in animal studies [3]. In addition, previous studies on Pycnogenol effects on human skin indicate that Pycnogenol supplementation may be reflected by concomitant changes at the molecular level. We were particularly interested in the in situ transcriptional expression of genes involved in the de novo synthesis of hyaluronic acid and collagen, as this would be of direct relevance for skin hydration, elasticity and firmness.

**Materials and Methods**

**Materials**
Pycnogenol capsules containing 25 mg of Pycnogenol were provided by Horphag Research, Geneva, Switzerland. The composition of the Pycnogenol capsule used in this study is shown in table 1.

**Volunteers**
Approval had been obtained from the Ethics Committee of the Heinrich Heine University Düsseldorf. The study was conducted according to the ethical rules stated in the Declaration of Helsinki Principles, and the ICH GCP guidelines were adhered to, as applicable. Twenty healthy postmenopausal women were enrolled after written informed consent. Their ages ranged from 55 to 68 years, and all individuals were non-smokers, had normal eating habits and no history of any skin disease.

**Nutritional Supplementation**
Figure 1 illustrates the study design. During the first visit after enrolment, demographic data were obtained and a nutrition questionnaire was filled out. After a wash-out phase of 8 days, all volunteers were supplemented with 3×25 mg Pycnogenol daily for a period of 12 weeks. At the beginning, after 6 (day 49) and 12 (day 91) weeks of supplementation, skin-physiological parameters were assessed as described below. In addition, at the beginning and after 12 weeks each time one 4-mm punch biopsy was obtained from buttock skin.

**Skin-Physiological Measurements**
All skin-physiological measurements were carried out by the same investigator in an air-conditioned room (room temperature 18–22°C, air humidity approx. 30–50%). To measure skin hydration, a Corneometer CM 825 (Courage-Khazaka Electronics GmbH, Cologne, Germany) was used. Skin elasticity was determined by means of a Cutometer MPA 580 (Courage-Khazaka...
Electronics GmbH). Skin topography was evaluated with the Vi-
sioscan® VC 98 (Courage-Khazaka Electronics GmbH). Echoc-
graphic evaluations were carried out with a 20-MHz B Scanner
(Dermascan C, Cortex Technology, Denmark). Assessments were
made according to the EEMCO guidelines.

Assessment of Gene Expression in Skin Biopsies

Biopsies were snap frozen in liquid nitrogen and stored at
–80°C until further analysis. For assessment of gene expression,
total DNA was extracted from frozen biopsies and gene expres-
sion measured by semiquantitative reverse-transcriptase PCR
(RT-PCR) as previously described [13]. In brief, for isolation of
RNA from frozen skin biopsies, 600 μl lysis buffer from a Peq-
Gold Total RNA Kit (PeqLab, Erlangen, Germany) was added,
and the samples were disrupted in a MixerMill MM300 (Retsch,
Haan, Germany) 3 times for 3 min with 30 Hz. Fifty nanograms
RNA were used for cDNA synthesis. PCR reactions were per-
formed in an Opticon 1 (MJ Research, Waltham, Mass., USA) us-
ing Sybr QPCR Supermix w. Rox (Invitrogen, Karlsruhe, Germa-
y). PCR conditions were as follows: activation of hot start Taq
polymerase at 94°C for 15 min; denaturation at 95°C for 20 s; an-
nealing at 55°C for 20 s; extension at 72°C for 30 s. Each sample
was subjected to PCR in duplicate using the appropriate primer
pairs for 45–50 cycles. For comparison of relative gene expression
the 2−ΔΔCt method was used [14]. Primer pairs used for RT-PCR
are shown in table 2.

Statistical Analysis

The Wilcoxon signed-rank test as a non-parametric test for the
comparison of differences between measurements as well as the t
test were used for statistical analysis (PASW Statistics 18), and p
values of less than 0.05 were considered statistically significant.

Results

Pycnogenol supplementation was well tolerated by all
volunteers. Skin hydration increased in the whole study
population by 8% after 6, but not after 12 weeks (fig. 2a).
This increase was even more pronounced, if volunteers
with dry skin were analysed separately. In this subgroup
(n = 13), a significant (p < 0.05), i.e. 21%, increase in skin
hydration was observed (fig. 2b). In general, increased skin hydration corresponds to a loss of echogenicity of
human skin, as can be demonstrated by high-frequency
(20-MHz) sonography as a reduction in pixel numbers.
Accordingly, in the present study the acoustic (= echo)
density of skin decreased under Pycnogenol supplemen-
tation by 2% in all volunteers and by 2.8% in volunteers
with dry skin (p < 0.05; data not shown).

Skin visco-elastic measurements confirmed the previ-
ous observation that Pycnogenol supplementation im-
proves skin elasticity [12]. In the evaluation with the Cu-
tometer, R2 and R7 values are the most important param-
eters, i.e. the closer they are to 1 (= 100%), the more

Table 2. Primer pairs for reverse-transcriptase PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer pair</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA (housekeeping gene)</td>
<td>5′-GCCGCTAGAGGTGAAATTCTTG-3′ 5′-CTTGGCTGGCAAAAGCTTTGCG-3′</td>
<td>15</td>
</tr>
<tr>
<td>COL1A1</td>
<td>5′-CTTGGGCTGACCCACCTCTCT-3′ 5′-CCGACATGCTTGTGCTCTT-3′</td>
<td>16</td>
</tr>
<tr>
<td>COL1A2</td>
<td>5′-GATTTGAGACCCCTCTTACCTCCTGAA-3′ 5′-GGTTGCTGGAGTCCTCAAGCTCA-3′</td>
<td>17</td>
</tr>
<tr>
<td>HAS-1</td>
<td>5′-GCAGGCTTTTGCTAGAGCTACT-3′ 5′-AACTGCTGCAAGGGTTATTCCTATAT-3′</td>
<td>18</td>
</tr>
</tbody>
</table>
Effect of Pycnogenol on Human Skin

Fig. 2. Skin hydration. At study start and again after 6 (day 49) and 12 (day 91) weeks of Pycnogenol supplementation, skin hydration was assessed with a Corneometer CM 825 (Courage-Khazaka Electronics GmbH). Presented are the mean values ± SE of the study volunteers. The statistical significance was obtained by t test, and p values less than 0.05 were considered statistically significant. Skin hydration increased in the whole study population (n = 20) by 8% after 6, but not after 12 weeks (a). This increase was even more pronounced when volunteers with dry skin were analyzed separately (b). In this subgroup (n = 13), a significant (p < 0.05), i.e. 21%, increase in skin hydration after 6 weeks was observed.

Discussion

In the present study we provide for the first time molecular evidence that nutritional supplementation with Pycnogenol may benefit human skin. We are aware that the present study is limited by its uncontrolled design, which we have chosen for ethical reasons to limit the number of biopsies by leaving out a placebo group. A well-designed placebo-controlled study with validated outcome variables such as hydration and skin elasticity could further substantiate the observed effects of Pycnogenol on human skin and its clinical relevance to treat symptoms of intrinsic aging such as xerosis, laxity and wrinkling but also environmentally induced detrimental changes of skin physiology [22, 23].
Under the conditions of an uncontrolled design, we found that supplementing volunteers with Pycnogenol for 12 weeks increased the expression of HAS-1. The enzyme HAS-1 is critically involved in the de novo synthesis of hyaluronic acid and thereby profoundly contributes to skin hydration and skin elasticity. Hyaluronic acid is synthesized by keratinocytes and fibroblasts [24]. The synthesis of this macromolecular glycosaminoglycan is accomplished by 3 different HA synthase isoforms (HAS-1, HAS-2 and HAS-3) [25] and the mRNA expression is dependent on cell type and modulated by cell density and various growth factors [26]. HAS-1 mRNA is expressed by both dermal fibroblasts [27] and epidermal keratinocytes [27, 28], and, thus, Pycnogenol may stimulate hyaluronic acid synthase in both cell types.

Hyaluronic acid, also referred to as hyaluronan, is a widely distributed glycosaminoglycan and an essential component of the extracellular matrix. Hyaluronan is involved in a variety of biological processes, such as maintenance of tissue architecture, cell proliferation, migration, differentiation, angiogenesis, wound healing and tumorigenesis [29–31]. The skin contains about half of the total-body hyaluronan [29] which distributes to the dermis and epidermis [32]. Large quantities of hyaluronic acid reside in the dermal connective tissue; in the epidermis, it is strongly expressed around the basal and spinous cells, whereas the terminally differentiated cells of the stratum corneum usually lack hyaluronan [27, 33]. We have previously shown [24] that chronic, repetitive UVB irradiation caused marked loss of hyaluronan from the papillary dermis because of transcriptional downregu-
tion of HAS-1, HAS-2 and HAS-3. Pycnogenol may thus represent a novel strategy to counteract photo-aging of human skin.

The present study shows that Pycnogenol induces HAS-1 mRNA expression and thus provides a mechanistic explanation for the previous observation that Pycnogenol supplementation may increase skin elasticity [12], as demonstrated by skin biophysical measurements. We confirm this observation and additionally demonstrate that volunteers with dry skin preferentially profit from intake of this nutritional supplement. Why average skin hydration was lower after 12 weeks than it was after 6 weeks is difficult to interpret, but may result from seasonal changes occurring during the trial period in summer. Accordingly, patients with some skin conditions, such as atopic dermatitis, may markedly benefit from Pycnogenol supplementation. The precise mechanism by which Pycnogenol induces HAS-1 mRNA expression currently remains unknown.

In addition to HAS-1 mRNA expression, COL1A1 and COL1A2 mRNA levels were increased, indicating that Pycnogenol supplementation may stimulate collagen de novo synthesis in human skin. This effect did not reach statistical significance which is most likely due to the relatively low number of volunteers who were assessed in the present study. Nevertheless, we believe that the observed effect is real because (i) it has previously been reported that Pycnogenol supplementation increases skin elasticity and (ii) we have observed in the present study that Pycnogenol supplementation, as assessed by Visioscan, reduces skin wrinkles by 3% and increases skin smoothness by 6% (data not shown).

In conclusion the present study confirms at a molecular level the beneficial effects Pycnogenol supplementation may provide to human skin. Our study indicates that Pycnogenol supplementation improves skin hydration and elasticity by inducing the de novo synthesis of hyaluronic acid. In addition, we provide some evidence that collagen de novo synthesis may be stimulated. The latter observation should prompt further studies to more closely evaluate the potential of Pycnogenol supplementation to counteract human skin aging.

Disclosure Statement

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