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Article

The *in vivo* effect of L-arginine on skin elasticity in mice

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The human skin aging process is a complex mechanism that can be induced both by intrinsic and extrinsic factors. Observations include a decrease in the biosynthetic and proliferative capacity of cells, increased expression of matrix metalloproteinases, reduction in collagen type I expression, and the progressive disappearance of elastic tissue in the papillary dermis. L-arginine, the substrate of nitric oxide synthesis, is involved in angiogenesis and cell proliferation, as well as an indirect precursor of collagen synthesis via the proline pathway. The aim of this study was to examine the tensile strength, histology, and immunohistochemistry of female and male mice skin receiving different concentrations of topically applied L-arginine, in order to evaluate the possibility of using L-arginine as an active cosmetic ingredient in antiaging products. The results suggest that the application of L-arginine improves the mechanical resistance of skin from older female mice (20 weeks old) and promotes the formation of a larger amount of collagen and elastic fibers in the skin when applied at a concentration of 15%.

Keywords: L-arginine. L-arginine/*in vivo* efficacy. Skin aging. Elastin. Collagen. iNOS.

INTRODUCTION

Like all organs, skin undergoes chronological aging, but unlike others, it is in direct contact with the environment, and therefore further ages as a result of environmental damage (Fisher *et al*., 2002; Pageon *et al*., 2007; Hwang, Yi, Choi, 2011; Levakov *et al*., 2012). The aging of human skin is a complex process, induced by both intrinsic factors resulting from tissue degeneration that are largely genetically determined, and by extrinsic factors caused by environmental exposure, predominantly including ultraviolet radiation, smoking, excessive alcohol drinking, and malnutrition. Among the external factors, sun exposure is considered the most harmful to skin (Naylor, Watson, Sherratt, 2011; Jenkins, 2002; Baumann, 2007; Levakov *et al*., 2012; El-Domyati, Medhat, 2015; Kammeyer, Luiten, 2015).

In intrinsic or chronological aging, changes in the skin are similar to those that occur in most of the internal organs. The changes occur partly because of endogenous injury accumulation due to the continuous formation of reactive oxygen species (ROS), which are generated by cellular oxidative metabolism (Jenkins, 2002; Tobin, 2017). Although the stratum corneum remains relatively unchanged throughout the aging process, the epidermis and dermis undergo a flattening of the dermal-epidermal junctions and a reduction in the proliferative and biosynthetic capability of skin cells, especially fibroblasts. This results in a decrease in the dermal matrix production and increased expression of matrix metalloproteinases (MMPs) (Jenkins, 2002; Oriá *et al*., 2003; Langton *et al*., 2010; Levakov *et al*., 2012; Tobin, 2017).

The main molecular components involved in the skin aging process are collagen, elastic fibers, and glycosaminoglycans. Among them, collagen is the most abundant extracellular component in skin that imparts the dermis tensile properties (Naylor, Watson, Sherratt, 2011; Jenkins, 2002). Collagen molecules are initially synthesized as a precursor, procollagen, that is post-translationally hydroxylated by prolyl hydroxylase, generating hydroxyproline residues that are required for the formation of collagen. Together, hydroxyproline and proline residues correspond to 23% of the collagen molecule (Bellon *et al.,* 1987; Barbul, 2008; Wu *et al*., 2011; Pokidysheva *et al*., 2013). Proline used for collagen biosynthesis in fibroblasts may be derived from glutamine,

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glutamate, ornithine, or arginine (Wu *et al*., 2011).

Previous studies have detailed the relationship between arginine, collagen synthesis, and deposition of collagen in wound-healing processes (Albina, Abate, Mastrofrancesco, 1993; Soneja, Drews, Malinski, 2005). Specifically, research has demonstrated that the proline pool size determines the rate of collagen synthesis, and the local synthesis of proline from their metabolic precursors, such as ornithine, arginine, glutamate and glutamine, is strengthened in some circumstances, apparently to compensate for a relative deficiency in preformed proline residues (Barbul, 2008).

L-arginine is a substrate for nitric oxide synthesis; therefore, it is indirectly involved in many important regulatory mechanisms, such as cell proliferation and angiogenesis (Shi *et al.,* 2003; Durante, 2013). L-arginine is metabolized by nitric oxide synthase (NOS) to nitric oxide (NO) and L-citrulline, or is metabolized to urea and ornithine by arginase-1 in the liver urea cycle. Ornithine, through the action of ornithine-γ-aminotransferase is converted to pyrroline-5-carboxylate, that is then metabolized to L-proline by pyrroline-5-carboxylate reductase. L-proline is required for the synthesis of many structural proteins, including collagen (Barbul, 2008; Durante, 2013).

Arginase-1 has been assumed to participate in wound-healing processes because it affects local cell proliferation and possibly collagen metabolism (Shi *et al*., 2003). Arginine supplementation in the diet of humans and rodents has a significant effect on the wound-healing process. Rats treated with L-arginine display increased tensile strength of scar skin and higher collagen deposition in comparison with controls (Seifter et al., 1978 *apud* Soneja, Drews, Malinski, 2005).

Therefore, the objective of this work was to assess the effect of the topical administration of L-arginine on female and male mouse skin elasticity to evaluate the possibility of its use as a cosmetic active ingredient.

MATERIAL AND METHODS

In vivo efficacy of L-arginine in improving mouse skin elasticity

To assess the effect of L-arginine on skin elasticity, Swiss female and male mice weighing between 20 and 25 g each were grouped according to age and sex. Group 1 comprised of female mice at 4–6 weeks old; group 2, female mice at 11–13 weeks old; group 3, female mice at 20 weeks old; group 4, male mice at 4–6 weeks old; group 5, male mice at 11–13 weeks old; and group 6, male mice at 20 weeks old. The mice groups were housed in plastic cages under controlled light (12 h light/dark cycle) and temperature (25 °C) conditions with water and food *ad libitum*. To evaluate the *in vivo* efficacy of L-arginine in improving skin elasticity, the mice received a topical application of 0.5 g of L-arginine dispersed in glycerol at different concentrations, or glycerol only (vehicle control group, Glyc.) for 15 days, or received no treatment (control group, CTRL). After the treatment period, the mice were euthanized in a carbon dioxide chamber. The abdominal skin of the mice was then shaved and samples of approximately 3 cm were removed. The fragments of removed tissue were either fixed in buffered formalin and processed for histological evaluation or used immediately to evaluate skin elasticity. All animal procedures were performed according to the rules of CONCEA (The Brazilian National Council for the Control of Animal Experimentation) and carried out under the approval of the Ethics Committee on Animal Use at the University of Brasilia (process number: 47217/2009). All efforts were made to minimize animal suffering.

Skin elasticity evaluation

The tissue samples that were removed from the mice were kept moist by the addition of saline solution. Skin elasticity was evaluated by measuring tensile strength using VersaTest® equipment coupled with a dynamometer. Skin samples were stretched until rupture and the maximum traction force tolerated by the tissue was determined.

Histological and immunohistochemical evaluation

Skin fragments from treated mice were immersed in 10% formaldehyde for 48 h, followed by use in routine histological procedures for paraffin embedding. Histological cross sections were made through the major axis of the skin fragments. Staining techniques used include hematoxylin and eosin (H&E) staining for the morphological analysis of tissue, Masson's trichrome staining for the analysis of collagen fibers (Hotchkiss, 1948), and Verhoeff's staining for the observation of elastic fibers (Verhoeff, 1908). The sections were examined by light microscopy and analyses were based on the histological characteristics of the tissue in comparison with the control group.

Tissue sections were also immunohistochemically stained using the immunoperoxidase technique in order to analyze inducible NOS (iNOS) expression, as described in the Spring Bioscience and Biogen protocol. The polyclonal antibody rabbit anti-iNOS (Spring Bioscience,

code E3740), was diluted in antibody diluent (Spring Bioscience, code ADS-125) at ratio of 1:100. The polymer used was Histofine® (Nichirei, Cod. 414341F) and the chromogen substrate was 3,3'-diaminobenzidine (DAB; Spring Bioscience, code DAB-125). Mice lung sections were used for positive and negative controls. For negative control staining, all steps were carried out except for the application of the primary antibody.

The results were expressed as the staining intensity scored with a semiquantitative ordinal scale (0–30%, 30-70%, and 70-100%) according to analyses by three blinded observers, and the mean was used as the final score.

Statistical Analyses

The obtained results were statistically analyzed by a variance test (*one-way* ANOVA) followed by Bonferroni, Dunnett, or Tukey multiple comparisons tests.

RESULTS

Skin elasticity evaluation

Skin samples were evaluated from mice that were treated with different concentrations of L-arginine suspended in glycerol, and with glycerol only (Glyc.) or that received no treatment (CTRL) to determine the skin's resistance to mechanical force using the VersaTest® equipment coupled to a dynamometer (Figure 1 and 2).

Figure 1 (A to C) presents the results obtained for the evaluation of the mechanical tensile strength of skin from female mice of different ages treated with different concentrations of L-arginine. For mice from group 3 (Figure 1C), treatment with L-arginine at any of the tested concentrations resulted in a significant increase in the tensile strength needed to disrupt the skin. For mice from group 2 (Figure 1B), a significant difference was observed between the control group and the group treated with L-arginine at 10% that showed an increase in elasticity. The mechanical resistance of skin samples from group 1 mice (Figure 1A) was significantly lower in the control group than in skin from all other groups that were treated with L-arginine or with the vehicle control. Generally, across all groups, there was a trend towards increased mechanical tensile strength of skin treated with L-arginine at 5% in comparison with controls, and decreased tensile strength for higher concentrations of L-arginine.

Figure 2 shows the results obtained for the treatment of male mice. A significant difference in the tensile force required to rupture the skin of older male mice treated with

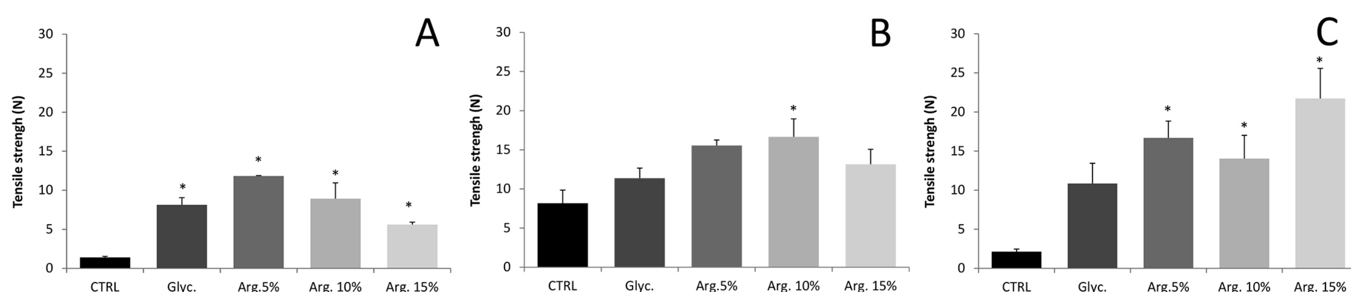

FIGURE 1 - Evaluation of the mechanical tensile strength of female mouse skin. (A) Group 1, (B) group 2, and (C) group 3. The mice were treated with glycerol only (Glyc.), dispersions of L-arginine in glycerol at 5% (Arg. 5%), 10% (Arg.10%), or 15% (Arg. 15%), or received no treatment (CTRL). $P < 0.05$; * *versus* CTRL; ($3 \leq n \leq 5$).

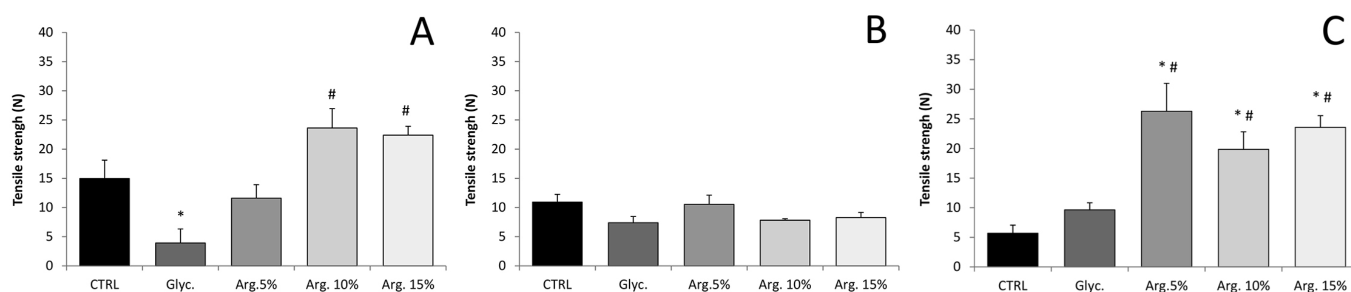

FIGURE 2 - Evaluation of the mechanical tensile strength of male mice skin. (A) Group 4, (B) group 5, and (C) group 6. The mice were treated with glycerol only (Glyc.), dispersions of L-arginine in glycerol at 5% (Arg. 5%), 10% (Arg.10%), or 15% (Arg. 15%), or received no treatment (CTRL). $P < 0.05$; * *versus* CTRL, # *versus* Glyc.; ($3 \leq n \leq 5$).

L-arginine was observed for all concentrations (Figure 2C). Skin of male mice at 11–13 weeks old displayed no significant difference following treatment (Figure 2B). The experiments performed on the younger male mice revealed that the skin strength of untreated were significantly greater than the skin strength of vehicle control group mice, and significantly lower in the mice of the vehicle group than the groups treated with L-arginine dispersions at concentrations of 10% or 15% (Figure 2A).

Histological evaluation

Evaluation of collagen fibers

The skin samples underwent routine histological examination and were stained by the Masson’s trichrome method to observe collagen fibers. Results were expressed as the staining intensity of fibers scored by three blinded observers, and the mean values for female and male mice are listed in Table I and II, respectively.

TABLE I - Evaluation of collagen fibers stained in female mouse skin

Group		Staining intensity
Group 1	CTRL	S > 70%
	Glycerol	30% < S < 70%
	Arg. 5%	S > 70%
	Arg. 10%	30% < S < 70%
	Arg. 15%	S > 70%
Group 2	CTRL	0 < S < 30%
	Glycerol	30% < S < 70%
	Arg. 5%	30% < S < 70%
	Arg. 10%	S > 70%
	Arg. 15%	0 < S < 30%
Group 3	CTRL	30% < S < 70%
	Glycerol	S > 70%
	Arg. 5%	30% < S < 70%
	Arg. 10%	30% < S < 70%
	Arg. 15%	S > 70%

The semiquantitative analysis of collagen fibers was performed by three blinded observers. S, mean score of staining.

Table I shows the results for collagen staining of female mouse skin by the Masson´s trichrome method. For older mice, L-arginine treatment at higher concentrations produced the best results in comparison with controls, while for younger mice, lower concentrations of L-arginine produced results similar to those for higher concentrations. For male mice (Table II), L-arginine treatment did not stimulate an increase in collagen fiber abundance. A selection of the photomicrographs that illustrate the analysis are shown in Figures 3 and 4.

TABLE II - Evaluation of collagen fibers stained in male mouse skin

Group		Staining intensity
Group 4	CTRL	0 < S < 30%
	Glycerol	S > 70%
	Arg. 5%	0 < S < 30%
	Arg. 10%	0 < S < 30%
	Arg. 15%	S > 70%
Group 5	CTRL	S > 70%
	Glycerol	0 < S< 30%
	Arg. 5%	S > 70%
	Arg. 10%	S > 70%
	Arg. 15%	S > 70%
Group 6	CTRL	S > 70%
	Glycerol	S > 70%
	Arg. 5%	0< S< 30%
	Arg. 10%	0 < S < 30%
	Arg. 15%	0 < S < 30%

The semiquantitative analysis of collagen fibers was performed by three blinded observers. S, mean score of staining.

Assessment of elastic fibers

Skin samples were stained by the Verhoeff method for the evaluation of elastic fibers. The obtained photomicrographs were analyzed by counting the fibers in three distinct and random fields of a checkerboard lattice with an area of 0.01 mm^2 shared into 100 equal parts (Figure 5). For older mice, the treatment with L-arginine at 15% resulted in an increase in the amount of elastic fibers in comparison with controls that received the glycerol vehicle only. For younger mice, the treatment did not stimulate any increase in the amount of elastic fibers. The photomicrographs illustrate the difference in the amount of elastic fibers in the skin of younger and older mice, as well as between older control and 15% L-arginine treated mice (Figure 5).

The difference in the amount of elastic fibers observed among mice of different ages that was observed in female mice was not observed in male mice (Figure 6). Nonetheless, the L-arginine treatment of male mice 11–13 weeks old was effective in stimulating the production of elastic fibers.

Figure 7 depicts the difference in the amount of elastic fibers in untreated skin from male and female

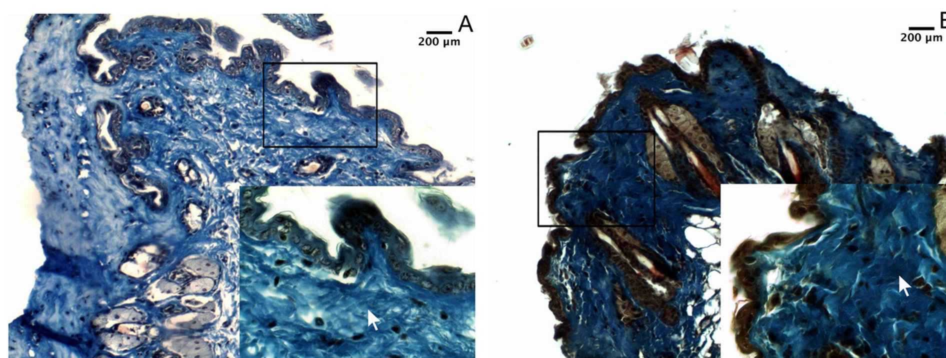

FIGURE 3 - Photomicrographs of skin stained with Masson's trichrome to observe collagen fibers (x100, details x400). (A) Female mouse skin from group 3 treated with vehicle and (B) female mouse skin from group 2 treated with L-arginine 10%.

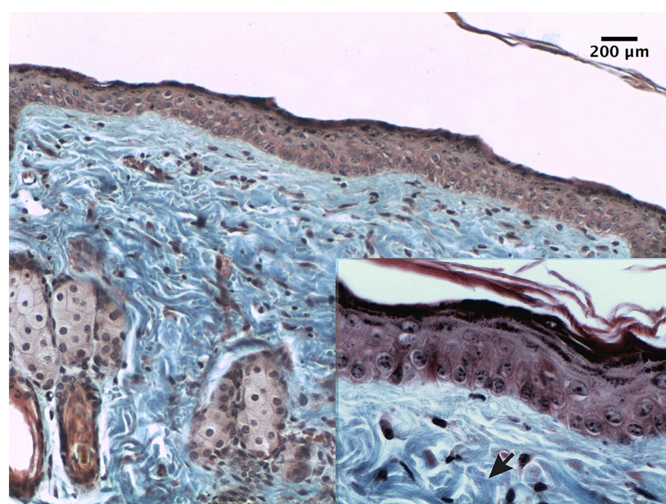

FIGURE 4 - Photomicrograph of male mouse skin from group 6 (CTRL) stained with Masson's trichrome to observe collagen fibers (x100, detail x400).

mice with age. For female control mice, the amount of elastic fibers decreases significantly with aging. This is in contrast with males that maintain a relatively uniform amount of elastic fiber across age groups, despite generally having less elastic fiber in the skin samples than the female mice.

Immunohistochemical detection of iNOS

The fragments of mouse skin were processed for histological analysis by immunohistochemical staining specific for iNOS detection. Results are expressed as the intensity and distribution of iNOS staining for female (Table III) and male (Table IV) mice.

The mouse skin samples from group 3 stained positive for iNOS, with staining intensity greater than 30% in the skin appendages for all treatments, including controls (Figure 8A, B, C). A slight positive staining for iNOS in the epidermis was only observed in CTRL group mice and those treated with L-arginine at 5% (Figure 8A, B). In group 2, only the skin of female mice that received treatment with L-arginine showed positive staining for iNOS. For group 1, female mice that received treatment with 5% L-arginine dispersion did not show iNOS staining in the skin.

For male mice, iNOS was not observed in most of the tissue processed by immunohistochemical detection except for in younger mice (group 4). More iNOS was expressed in the skin of older mice treated with 15% L-arginine (group 6) and in the skin of younger animals treated with 10% L-arginine (group 4, Table IV). Figure 9 illustrates the photomicrograph of skin from 4–6-week-old mice treated with L-arginine at 10%.

DISCUSSION

With increasing life expectancy, modern women can spend more than a third of life in the post-menopausal period. During this period, hormonal changes, such as the loss of estrogen production, accelerate changes in the collagen and elastic fibers in skin, resulting in increased wrinkling and sagging after menopause. Research has shown that approximately 30% of collagen is lost in the first 5 years of this period, and that the rate of collagen loss is 2.1% per year after menopause (Son *et al*., 2005, Phuong, Maibach, 2015).

Several substances have been tested to assess their contribution to delaying the skin aging process, by various mechanisms, such as scavenging of ROS and restoration of the redox balance (Hwang, 2010), inhibiting the expression or activity of MMP (Valenti *et al*., 2011), increasing the expression or regulation of tissue inhibitor of metalloproteinases (TIMP) (Landau, 2007), inhibition of elastosis (Langton *et al*., 2010), or simply by protecting against ultraviolet (UV) radiation that promotes the

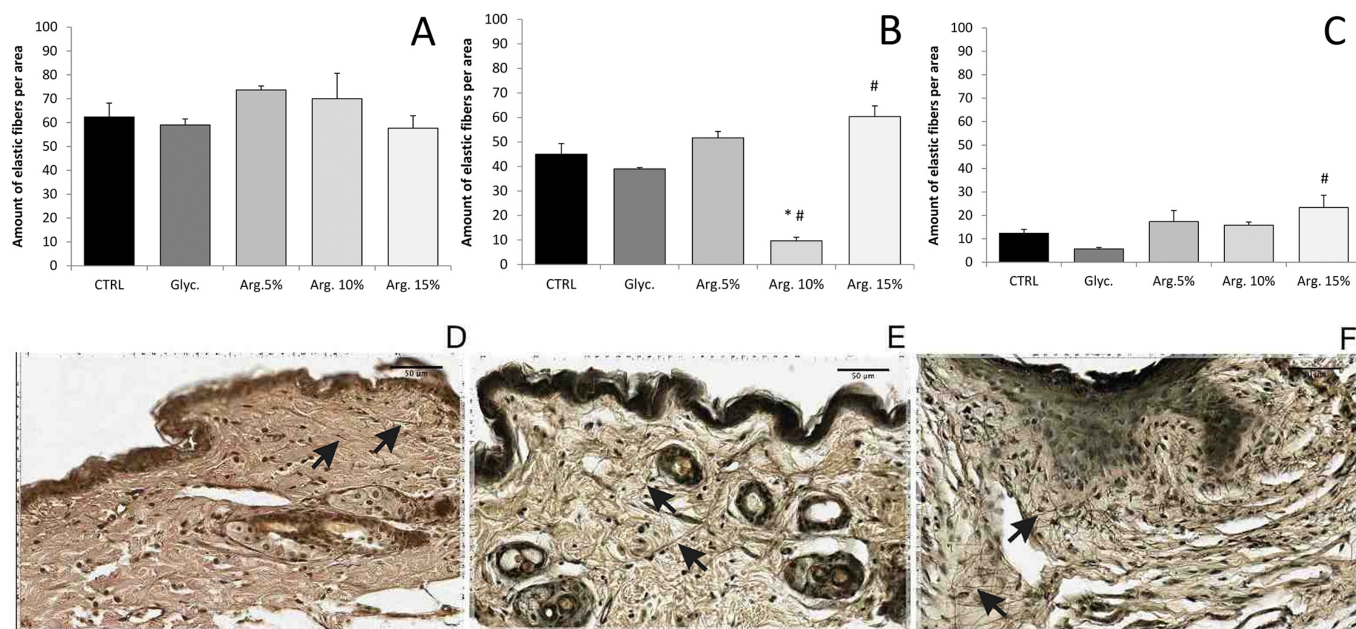

FIGURE 5 - Quantitative analysis of elastic fibers in female mouse skin. Mice from group 1 (A), group 2 (B), and group 3 (C) were treated with vehicle (Glyc.), L-arginine at 5% (Arg. 5%), 10% (Arg. 10%), or 15% (Arg. 15%), or did not receive any treatment (CTRL). $P < 0.05$, * *versus* CTRL, # *versus* Glyc.; ($3 \leq n \leq 5$). (D–F) show the photomicrographs (x400) of the skin sections stained for evidence of elastic fibers using the Verhoeff method: (D), mice from group 3, CTRL; (E), mice from group 3 treated with L-arginine at 15%; and (F), mice from group 1 treated with L-arginine at 10%.

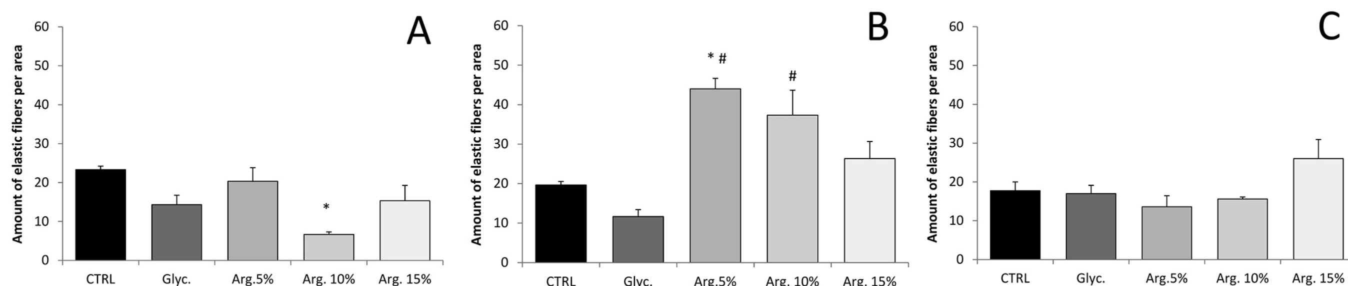

FIGURE 6 - Quantitative analysis of elastic fibers in male mouse skin. Mice from group 4 (A), group 5 (B), and group 6 (C) were treated with vehicle (Glyc.), L-arginine at 5% (Arg. 5%), 10% (Arg. 10%), or 15% (Arg. 15%), or did not receive any treatment (CTRL). $P < 0.05$, * *versus* CTRL, # *versus* Glyc.; ($3 \leq n \leq 5$).

formation of ROS and triggers many of these processes that are responsible for aging (Choe *et al*., 2003).

As already mentioned, Seifter and collaborators (Soneja, Drews, Malinski, 2005) described an increase in tensile strength from scar and collagen deposition in the healing process in rats fed the arginine-supplemented diet when compared with that in the control group, fed the arginine-free diet. In the present study, an increased resistance of the skin to traction force found when L-arginine dispersions were topically administered in not only groups 2 and 3 mice, but also in group 1. Collagen fiber analysis revealed an increase in the amount of fibers in the skin of mice from groups that received treatment with higher concentrations of L-arginine in comparison with that at lower concentrations. Estrogen deficiency may be responsible for skin aging at the start of the infertile period, since it affects the loss of collagen and skin water content (Calleja-Agius, Brincat, 2009; Phuong, Maibach, 2015), and it could explain the observed decrease in traction resistance and collagen in the skin of the older group of female mice (group 3). Once L-arginine is metabolized in the urea cycle, yielding ornithine that is used for proline production, which in turn is used for fibroblast collagen synthesis (Barbul, 2008), the amino acid may be contributing to the increase of elasticity and the amount of collagen fibers in the skin of treated mice.

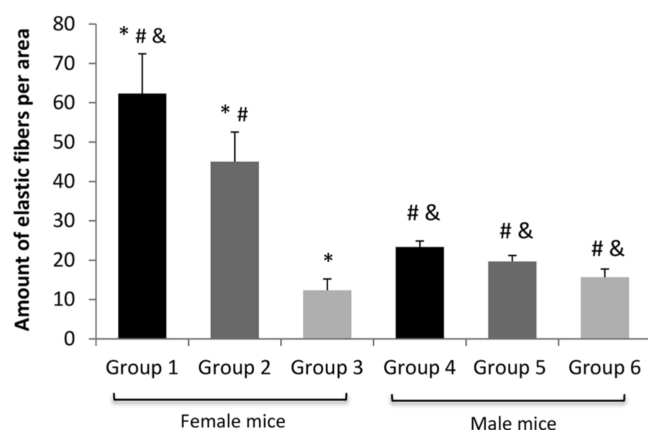

FIGURE 7 - Amount of elastic fibers in the skin of male and female mice. The mice did not receive any kind of treatment (CTRL). Elastic fibers were counted in three distinct and random fields of a checkerboard lattice with an area of 0.01 mm^2 shared into 100 equal parts. *, #, and &, indicate a significant difference between flagged groups, $P < 0,05$; n = 3.

The histological sections of skin stained with Masson's trichrome for collagen analysis revealed that the male mice from the CTRL group presented more collagen fibers in the skin than the female mice from the CTRL group. This feature was observed for all age groups, except for the youngest mice that had a high intensity of collagen fiber staining in the skin for both male and female mice. This age-dependent difference observed in the skin of females may be explained by estrogen deficiency that is characteristic of the final phases of childbearing age.

L-arginine can also be metabolized in the nitric oxide synthase cycle resulting in NO production. Some studies have shown that NO induces both expression and activation of MMP-1 in chondrocyte cultures, as well as induces MMP-1 and MMP-2 expression and activity in fibroblasts (Lin *et al*., 2003; Choe *et al*., 2003). Therefore, iNOS expression in mouse skin was evaluated by immunohistochemistry and the results show an agreement with this supposition, as the skin from group 1 mice treated with vehicle or 10% L-arginine had a lower amount of collagen fibers than skin treated with 15% L-arginine, which also did not exhibit iNOS expression in the epithelium or showed very little iNOS expression in the connective tissue. It is reasonable to assume that 10% L-arginine could induce NO production that leads to the induction of collagenase expression. Although several studies indicate that the synthesis of collagen can be inhibited by NO in several types of cells, such as arteriole, vascular smooth muscle, and mesothelial cells (Chatziantoniou *et al.*, 1998; Myers, Tanner, 1998; Owens, Milligan, Grisham*,* 1996), other studies have shown that NO induces collagen synthesis in fibroblasts in the lamina

TABLE III - Immunohistochemical iNOS staining of female mouse skin

Group		Epithelium	Cutaneous annexes	Connective tissue
Group 1	CTRL	0 < IS < 30%, U	30% < IS < 70%, U	30% < IS < 70%, U
	Glycerol	0 < IS < 30%, D	0 < IS < 30%, D	-
	Arg. 5%	-	-	-
	Arg.10%	IS > 70%, U	30% < IS < 70%, U	IS > 70%, U
	Arg.15%	-	0 < IS < 30%, D	0 < IS < 30%, D
Group 2	CTRL	-	-	-
	Glycerol	-	-	-
	Arg. 5%	*	*	*
	Arg.10%	30% < IS < 70%, U	0 < IS < 30%, U	0 < IS < 30%, D
	Arg.15%	30% < IS < 70%, U	30% < IS < 70%, U	0 < IS < 30%, D
Group 3	CTRL	0 < IS < 30%, D	30% < IS < 70%, U	30% < IS < 70%, D
	Glycerol	-	30% < IS < 70%, U	-
	Arg. 5%	30% < IS < 70%, U	30% < IS < 70%, U	0 < IS < 30%, D
	Arg.10%	-	30% < IS < 70%, U	-
	Arg.15%	-	30% < IS < 70%, U	-

The intensity and distribution of marked iNOS in epithelial skin, cutaneous annexes, and connective tissue are listed. The values are the average of semiquantitative analyses made by three blinded observers. IS, immunohistochemical staining; -, absence of staining; *, insufficient material for analysis. The staining distribution was classified as diffuse (D), focused (F), or uniform (U).

TABLE IV - Immunohistochemical iNOS staining of male mouse skin

Group		Epithelium	Cutaneous annexes	Connective tissue
Group 4	CTRL	0 < IS < 30%, D	30% < IS < 70%, U	IS > 70%, U
	Glycerol	0 < IS < 30%, D	0 < IS < 30%, D	0 < IS < 30%, U
	Arg. 5%	0 < IS < 30%, D	0 < IS < 30%, D	0 < IS < 30%, D
	Arg.10%	IS > 70%, U	30% < IS < 70%, U	30% < IS < 70%, U
	Arg.15%	-	0 < IS < 30%, D	-
Group 5	CTRL	-	-	-
	Glycerol	-	-	-
	Arg. 5%	-	-	-
	Arg.10%	-	-	-
	Arg.15%	-	0 < IS < 30%, D	-
Group 6	CTRL	-	0 < IS < 30%, F	-
	Glycerol	-	0 < IS < 30%, F	-
	Arg. 5%	-	-	-
	Arg.10%	-	-	-
	Arg.15%	IS > 70%, U	IS > 70%, U	0 < IS < 30%, U

The intensity and distribution of marked iNOS in epithelial skin, cutaneous annexes, and connective tissue are listed. The values are the average of semiquantitative analyses made by three blinded observers. IS, immunohistochemical staining; -, absence of staining. The staining distribution was classified as diffuse (D), focused (F), or uniform (U).

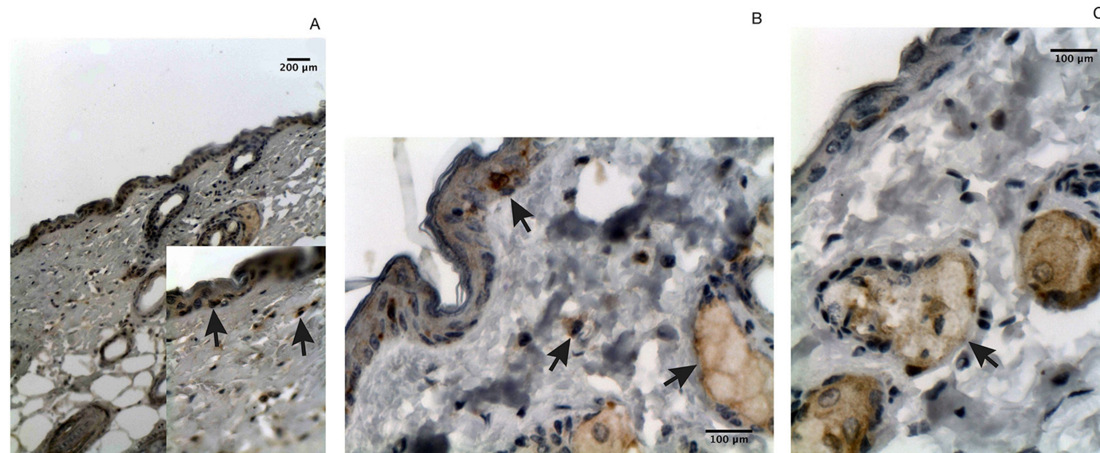

FIGURE 8 - Photomicrographs of immunohistochemical iNOS detection in female mouse skin. Immunohistochemical iNOS detection of group 3 mice. (A) control group, with positive staining in the connective tissue and skin appendages (x100, detail x400); (B) group treated with 5% L-arginine, showing positive staining in the epithelial cells, connective tissue, and skin appendages (x400); (C) group treated with L-arginine 15%, with positive staining only in cutaneous annexes (x400).

propria of the small intestine (Chakravortty, Kumar, 1997) and in the re-epithelialization of normal skin as part of the healing processes (Stallmeyer *et al*., 1999; Hsu *et al*., 2006).

Witte and coworkers (2000) revealed that although the NO donor used in the study presented a cytostatic effect on fibroblasts, the administration of low concentrations of NO donor in rat dermal fibroblast cultures enhanced collagen synthesis. The authors suggested that NO upregulates collagen synthesis in dermal fibroblasts without affecting collagen breakdown activity, acting by a posttranslational mechanism. L-arginine is a substrate either for arginase or for nitric oxide synthase. Arginase expression regulates arginine bioavailability for NO synthesis (Gobert *et al*., 2000; Kavalukas *et al.,* 2012), and despite the V_{max} of arginase for arginine exceeding that

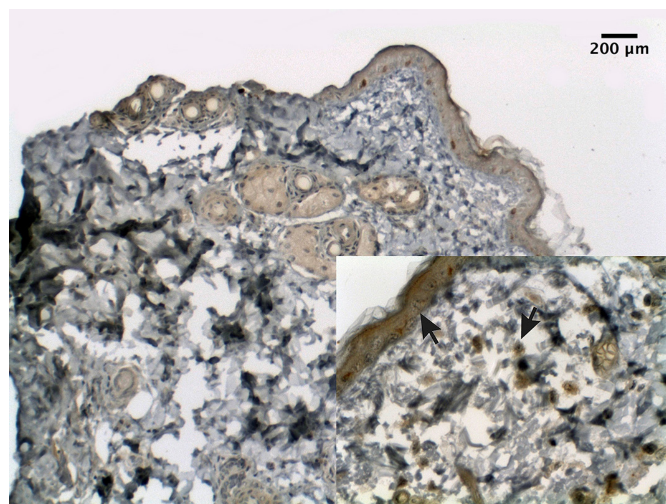

FIGURE 9 - Photomicrograph of immunohistochemical iNOS detection in male mouse skin. Skin of group 4 mice treated with 10% L-arginine marked by positive immunohistochemical iNOS staining in the epithelial cells, connective tissue, and cutaneous annexes (x100, detail x400).

of NOS by approximately 1000-fold, the affinity of NOS for arginine is greater (Kavalukas *et al*., 2012). Thus, in agreement with the results by Witte and coworkers (2000), the results of the present study also suggest that topically administrated arginine can penetrate into the skin, and owing to its higher affinity for NOS, it binds preferentially to NOS in the synthesis of NO, and thereby induces the synthesis of collagen.

Elastic fibers also undergo age-related changes. The fragmentation of elastic fibers results in a decrease in the physiological elasticity of the skin (Waller, Maibach, 2006). Furthermore, aged cells show a reduced ability to resynthesize elastic fibers that have degraded naturally (Jenkins, 2002). In the present study, a significant decrease in the amount of elastic fibers was found with aging in female mice, possibly explained by the estrogen deficiency characteristic of the final stages of childbearing age. Group 3 mice showed the largest amount of elastic fibers in the skin after treatment with L-arginine at 15% in comparison with mice from other groups. This suggests that L-arginine might contribute to an increase in the amount of elastic fibers in the skin of mice over 20 weeks of age, representing animals in the final stages of reproductive aging.

CONCLUSION

In the present study, topical treatment with L-arginine improved skin resistance to tensile force in 20-week-old female mice (group 3), as well as treatment with 15% L-arginine stimulated formation of larger amounts of elastic fibers and collagen. According to these results, L-arginine, which exhibits a hydrophilic character, could be a promising active ingredient in cosmetics to contribute to the postponement of skin aging effects, especially for women nearing the menopausal period.

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