

# Effective eye care using quinoa natural peptides

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In recent years, consumers are increasingly seeking cosmetic products that contain natural ingredients without compromising on efficacy. In the past four years, there has been a 52% increase in Beauty and Personal Care global product launches with “natural” claims.<sup>1</sup> To address this growing market demand, TRI-K Industries developed a unique NaturePep® natural peptide technology using sustainable botanical protein sources and natural superfoods. Superfoods are viewed as healthy, nutrient rich natural sources and appeal to consumers desire to live a healthy lifestyle.

NaturePep Quinoa (INCI name: Chenopodium Quinoa Seed Extract) was designed to address difficult to treat skin problems such as under eye bags, eyelid sagging, deep folds and creases. This natural technology represents an effective, sustainable, non-invasive solution to improve the eye contour area, which remains one of the most sought after anti-ageing categories.

NaturePep Quinoa is a superfood derived natural peptide technology obtained using sustainable vegetable Quinoa seed protein and a highly controlled proprietary manufacturing process, which allows the creation of a specific composition consisting of small natural peptide fragments with low average molecular weight. This specific composition ensures enhanced bioavailability to skin and a broader range of biological activity, resulting in multifunctional benefits. Chenopodium Quinoa Seed Extract is clinically proven to effectively reduce under eye bags and puffiness after 28 days of treatment. It also supports the structural integrity of the skin, reshaping the eyelid contour by smoothing the creases and lifting the folds.

## Causes of under eye bags and eyelid wrinkles

Skin around the eyes is very thin and fragile, and is more prone to ageing compared to all other facial areas.<sup>2</sup> The skin on the eyelids is the thinnest of the body (0.5 mm), has little subcutaneous fat and is more prone to loss of elasticity and firmness.<sup>2,3,4</sup> Eyelid skin shows the state of health and

### Abstract

NaturePep®Quinoa is part of TRI-K Industries' NaturePep natural peptide technology and addresses difficult to treat skin problems such as under eye bags, puffiness, eyelid sagging, folds and creases. Due to a multi-targeted mechanism of action, this unique bioactive reduces the under eye bags by stimulating adipocyte lipolysis. It also stimulates the production of extracellular matrix components to strengthen fragile skin around the eyes. The specific composition consisting of small natural peptide fragments ensures a broader range of biological activity and multiple skin benefits to de-puff, rejuvenate, lift and reshape the eye contour appearance. This versatile technology represents an effective natural anti-ageing solution for the cosmetic eye care category.

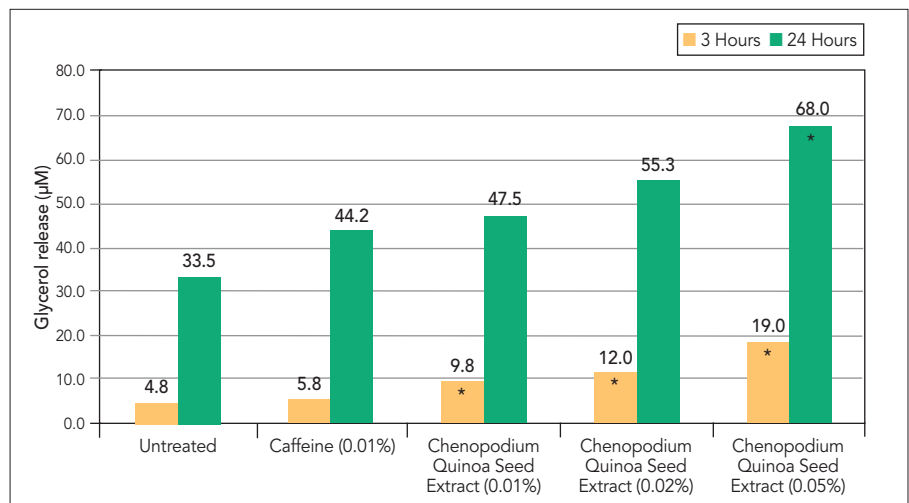


Figure 1: Average glycerol release from adipocyte cells treated with Chenopodium Quinoa Seed Extract, caffeine and untreated control after 3 hours and 24 hours of incubation.

Table 1: Statistically significant fold-change (FC) values and the percentage change in gene expression after Chenopodium Quinoa Seed Extract treatment compared to the control vehicle. Genes with the linear FC values of 1.5 or greater are considered statistically significant.

Gene ID	Gene Name	Biological function in skin	Linear Fold Change (FC)	% Change Gene Expression
CTGF	Connective tissue growth factor	Stimulation of ECM	2.28	128% increase
BMP2	Bone morphogenic protein 2	Stimulation of ECM; Stimulation of adipocyte lipolysis	2.14	114% increase
TGFβ1	Transforming growth factor beta 2	Stimulation of ECM. Cell renewal and regeneration	1.82	82% increase
FOXO3	Forkhead box O3	Anti-ageing. Protection against ROS	2.04	104% increase
CLDN7	Claudin 7	Stimulation of epidermal barrier	1.93	93% increase

age of an individual more rapidly than any other skin areas.<sup>2</sup>

There are several causes for under eye bags and puffiness:

Fluid retention which builds up mainly due to poor lymphatic circulation and increased capillary permeability.

Skin around the eyes and the supportive tissues weaken with age, collagen is decreased, skin becomes saggy and the normal fat that helps support the eyes moves and accumulates under the eyes as prominent bags and puffiness.<sup>2,3,4</sup>

### Methods

#### Gene expression assay

The effect of the Chenopodium Quinoa Seed Extract on gene expression that involves skin ageing has been evaluated on full thickness EpiDermFT™3D skin equivalent model (EFT-400 MatTek). Gene expression was analysed in the full thickness culture containing epidermal and dermal layers together. Gene expression assay has been performed using a qPCR array following 24 hour exposure to the cultured media treated with this natural ingredient (0.372% active) compared to a control vehicle.

The gene expression analysis showed statistically significant changes in the expression of several critical ageing genes. Table 1 shows statistically significant increases in the upregulation of several critical genes involved in aging processes compared to the control vehicle. The percentage change in each gene expression compared to the

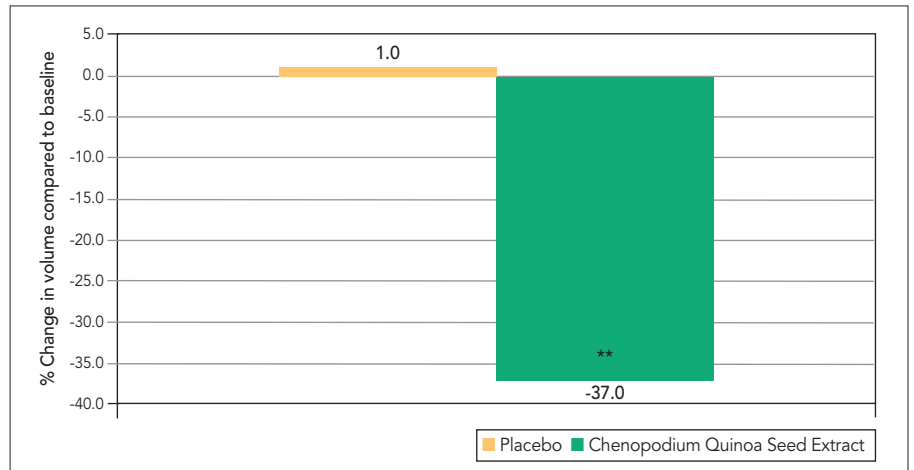


Figure 2: The % change in volume of under eye bags after 28 days of treatment with Chenopodium Quinoa Seed Extract and placebo creams compared to baseline.

control vehicle is also provided in Table 1.

Study results demonstrated that this natural ingredient upregulates the expression of CTGF, BMP2 and TGFβ1 growth factors, which stimulate the production of the extracellular matrix (ECM) proteins and improve the structural integrity of the dermis by modulating cellular migration and proliferation, stimulating cell-to-cell communication from keratinocytes to dermal fibroblasts via signalling and stimulating fibroblasts to synthesise ECM proteins.<sup>5,9</sup> It also upregulates CLDN7 gene expression to strengthen epidermal barrier function and FOXO3 transcription factor, which protects against extrinsic ageing by regulating

damaging reactive oxidative species, limiting free radical accumulation, and regulating protein homeostasis.<sup>10-13</sup> Chenopodium Quinoa Seed Extract treatment also upregulates the expression of BMP2 growth factor, which is reported to stimulate the adipocyte lipolysis.<sup>14, 15, 16</sup>

#### In vitro assay: adipocyte lipolysis

This *in vitro* study was conducted on adipocytes obtained from the eye area of a Caucasian female (53 years old). This study determines the ability of testing materials to induce lipolysis in cultured adipocytes. Glycerol release was measured as an index of lipolysis. The amount of free glycerol released was measured using a UV spectrometer, after 3 and 24 hours after incubation with tested materials (Fig 1). Facial adipocytes from the eye region were incubated with Chenopodium Quinoa Seed Extract at different concentrations, caffeine (benchmark) and untreated control. The higher the glycerol content released, the higher the ingredients ability to break down fats, thus to minimise the under eye bags. The *in vitro* results prove that Chenopodium Quinoa Seed Extract treatment stimulates the adipocyte lipolysis significantly better than the untreated control ( $p < 0.05$ ) and provides similar results versus caffeine.

#### Mechanism of action

This unique natural extract containing small peptide fragments demonstrates a multi-targeted biological mechanism of action and provides multiple skin benefits:

- Stimulates the adipocyte lipolysis and breaks down the fat under the eye, thus reducing the under eye bags. This is partially due to the vegetable carnitine formed in situ during our unique manufacturing process. Carnitine is known to stimulate adipocyte lipolysis.<sup>17</sup> Gene expression results show that this natural ingredient also upregulates BMP2 growth factor known to stimulate the adipocyte lipolysis.

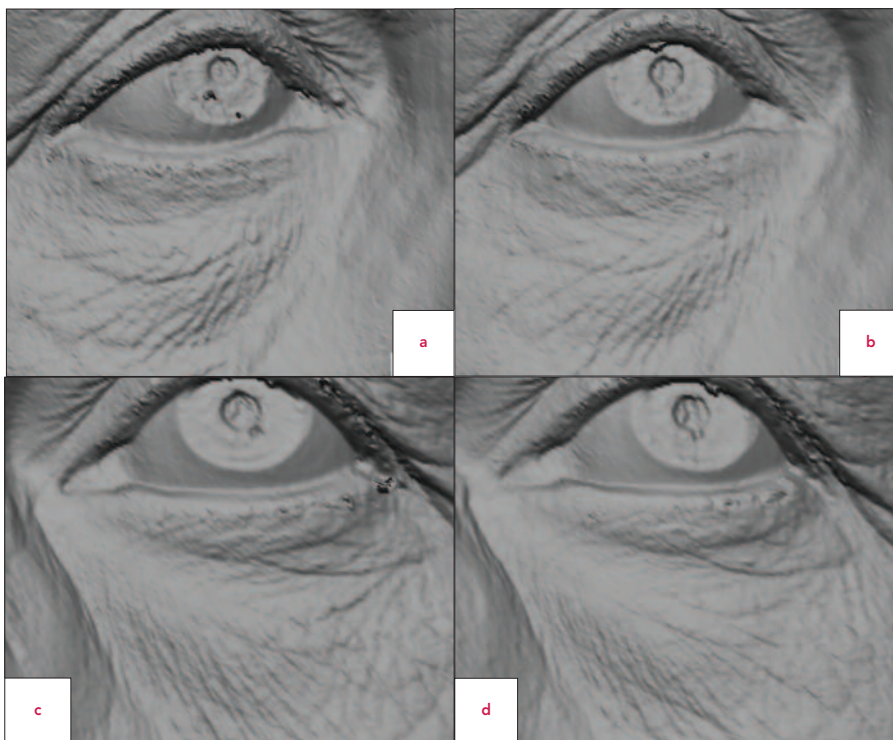
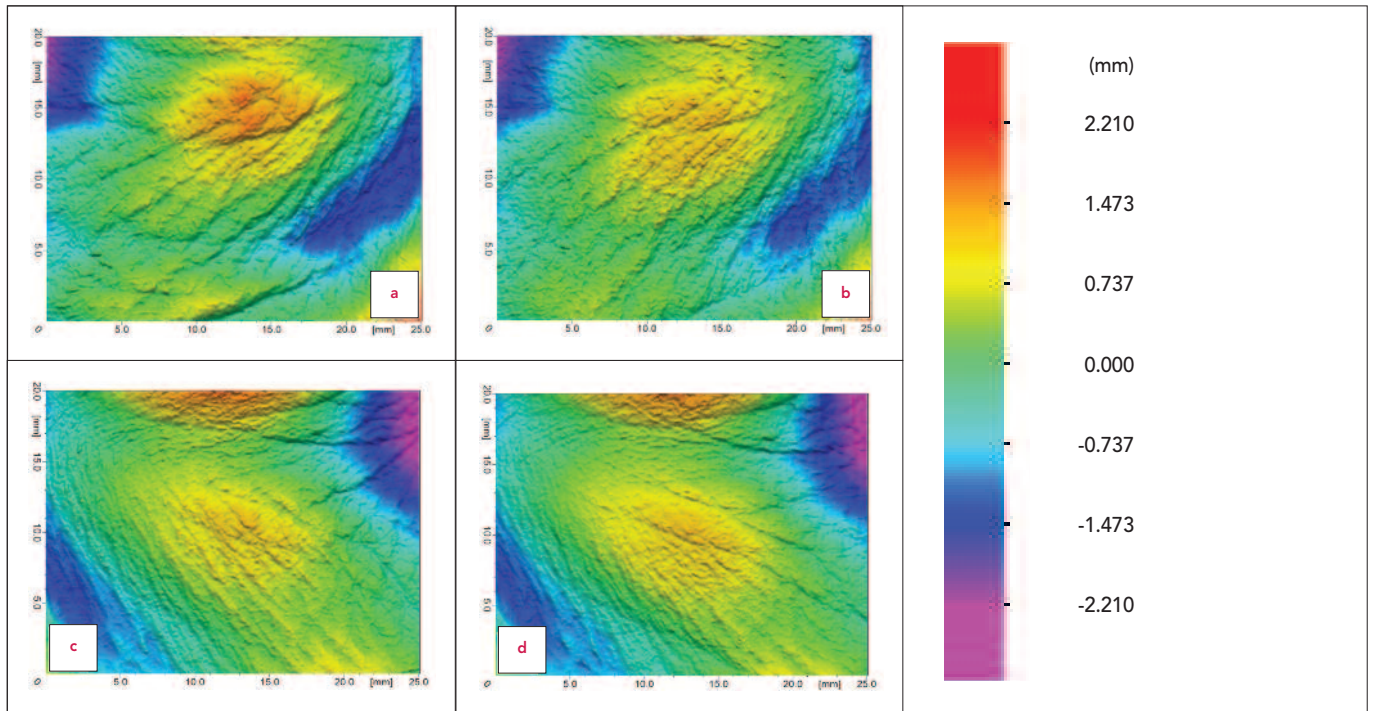


Figure 3: DermaTop® 3D pictures of a representative subject of 63 years old showing a 75% decrease in under eye bags and a smoother appearance of skin site treated for 28 days with Chenopodium Quinoa Seed Extract (Fig 3b) compared to baseline (Fig 3a). Lower pictures show placebo skin sites at baseline (Fig 3c) and after 28 days of treatment (Fig 3d).



**Figure 4:** 3D topographic images from the same subject (63 years old) showing reduction in under eye bags and smoother texture of skin site treated with Chenopodium Quinoa Seed Extract (Fig 4b) after 28 days of treatment compared to baseline (Fig 4a). Lower pictures show placebo skin sites at baseline (Fig 4c) and after 28 days of treatment (Fig 4d).

- Upregulates critical ageing growth factors such as CTGF, TGF- $\beta$ 1, BMP2 which stimulate the production of ECM proteins in dermis to help strengthen fragile skin from the eyelid area.
- Upregulates the CLND7 gene to help strengthen the epidermal barrier integrity.
- Upregulates the FOXO3 gene, which delivers anti-oxidant and anti-ageing benefits.

#### *In vivo* clinical study protocol

To evaluate the ability of the Chenopodium Quinoa Seed Extract to reduce under eye bags and eyelid creases, an *in vivo* clinical study was carried out on 16 female Caucasian subjects. All subjects (phototype I to III, age 45-65 years old) were selected to have under eye bags and deep eyelid folds at baseline. Subjects discontinued the use of any other cosmetic products on their face and used a cream containing Chenopodium Quinoa Seed Extract at 2% and a placebo cream in a dosage of 2.5mg/cm<sup>2</sup> twice a day application for 28 days. This was a single blind randomised hemi-face intra-individual *in vivo* clinical study.

#### *In vivo* measurements: under eye bags volume, eyelid average roughness and fold heights

Measurements were assessed directly *in vivo* using the fringe projection system DermaTOP® image analysis. This technique consists in calculating a phase image from acquired with interference fringe projection. The image allows determining the height of each point. The acquisition software allows

obtaining 2D and 3D measurements. In this study, we measured the eye bag volume (mm<sup>3</sup>), the eyelid average roughness (Ra) and eyelid fold height (Rz) at baseline (Day 0) and after 28 days of treatment (Day 28). A decrease in the volume of the under eye bags characterises an anti-puffiness effect. A decrease in the average roughness (Ra) indicates smoother skin and anti-crinkle effect on the eyelids. A decrease in the eyelid fold heights (Rz) characterises a lifting and reshaping effect of the product. Statistical analysis comparison between the groups was performed using paired - Student t test or a Wilcoxon paired ranks test using SAS 9.4 software. The significance level was set at  $p < 0.05$ .

### Results

#### Reduction of under eye bags and puffiness

A decrease in the volume of under eye bags characterises an anti-puffiness effect. The results demonstrate that after 28 days of treatment, Chenopodium Quinoa Seed Extract significantly decreased the volume of the under eye bags by 37% compared to baseline conditions ( $p < 0.01$ ) and by 38% compared to placebo ( $p < 0.01$ ). The percent change in the average volume of the under eye bags for the skin sites treated with Chenopodium Quinoa Seed Extract cream and placebo cream in comparison with baseline is illustrated in Figure 2.

DermaTOP® 3D pictures and 3D topographic coloured images of under eye bag area for a subject of age 63 at baseline and after 28 days of treatment with Chenopodium Quinoa Seed Extract and

placebo creams are shown in Figures 3 and 4.

This subject reports a 75% reduction in under eye bags compared to baseline after Chenopodium Quinoa Seed Extract treatment. As is evident from Figure 3b, under eye skin texture is visibly smoother and more rejuvenated after the treatment with this natural ingredient compared to baseline and placebo skin sites.

#### Reduction of eyelid roughness and fold heights

After 28 days of treatment Chenopodium Quinoa Seed Extract cream visibly decreased the average roughness (Ra) of the eyelids by 15% compared to the baseline conditions ( $p < 0.05$ ) and by 19% compared to placebo ( $p < 0.01$ ). This natural ingredient also decreased the eyelid fold heights by 13% compared to baseline ( $p < 0.01$ ) and performed significantly better compared to placebo ( $p < 0.05$ ) (Fig 5).

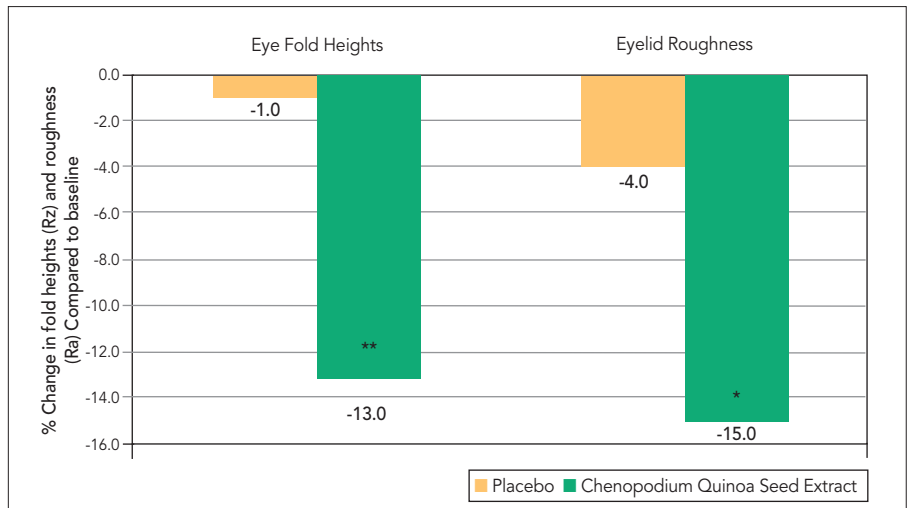
Figure 6 demonstrates significant reduction in eyelid roughness and folds for a representative subject of 57 years old. This subject shows a 45% reduction of eyelid roughness and a 29% reduction of folds compared to baseline after 28 days of treatment with this natural ingredient. As is evident from Figure 6d, eyelid folds are visibly more lifted after Chenopodium Quinoa Seed Extract treatment and the overall eyelid contour appears smoother, more rejuvenated and younger looking compared to baseline and placebo skin sites.

### Conclusion

NaturePep Quinoa, due to a multi-targeted



mechanism of action, visibly reduces under eye bags and puffiness by stimulating adipocyte lipolysis. Even more, it stimulates the production of extracellular matrix critical components in the dermis to help strengthen delicate eyelid skin and enhance epidermal barrier integrity for a healthier and more youthful eye area. The small natural peptide fragments ensure a wider range of biological activity and multifunctional benefits on the skin around the eyes to de-puff, rejuvenate, lift and reshape the eye contour appearance. Chenopodium Quinoa Seed Extract is a versatile technology that can be easily incorporated into a variety of eye care products and represents an effective natural anti-ageing solution for the cosmetic eye care category. PC



**Figure 5:** The % change in eyelid fold heights (Rz) and eyelid roughness (Ra) after 28 days of treatment with Chenopodium Quinoa Seed Extract and placebo creams compared to baseline.

**References**

- 1 Mintel GNPD Database (January, 2019).
- 2 Draelos ZD. Pigmentation, swelling and wrinkling in the eye area. *Anti-aging: Physiology to formulation book*: Allured Publication. 2006; 25-34.
- 3 Draelos ZD. *Cosmetics and Dermatological Problems and Solutions. Eyes. 3rd eds.* 2011; 11; 76-90.
- 4 Draelos ZD, Thaman LA. Cutaneous formulation issues. *Cosmetic formulation of skin care products.* 2006. 30; 3-26.
- 5 Sternlicht ON, Gerritsen M, Goldschmeding K. Could aging human skin use a connective

- tissue growth factor boost to increase collagen content? *Journal Investigative Dermatology.* 2010; 130: 338-341.
- 6 Quan T, He T, Kang S, Voorhees JJ, Fisher GJ. Connective tissue growth factor: expression in human skin in vivo and inhibition by ultraviolet radiation. *Journal of Investigative Dermatology.* 2002; 118 : 402-408.
- 7 Quan T, Shao Y, He T, Voorhees JJ, Fisher GJ. Reduced expression of connective tissue Growth Factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. *Journal of Investigative Dermatology.*

- 2010; 130: 415-424.
- 8 Varga J, Roosenbloom J, Jimenez SA. Transforming growth factor (TGF $\beta$ ) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. *Biochemistry Journal.* 1987; 247: 597-604.
- 9 Govinden R, Bhoola KD. Genealogy, expression and cellular function of transforming growth factor -  $\beta$ . *Pharmacology & Therapeutics* 2003; 98: 257-265.
- 10 Kim J, Choi, H., Chao E, Lee, TR. FoxO3a is an antimelanogenic factor that mediates antioxidant-induced depigmentation. *Journal of Investigative Dermatology.* 2014; 134: 1378-1388.
- 11 Kim J, Cho SJ, Kim SH, Cho D et al. Effects of Korean ginseng berry on skin antipigmentation and antiaging via FoxO3a activation. *Journal of Ginseng Research.* 2016; 1-7.
- 12 Mehul B, Bernard D, Schmidt R. Calmoduline-like skin protein: a new marker of keratinocyte differentiation. *J. invest Dermatol.* 2011; 116: 905-909.
- 13 Findley MK, Koval M. Regulation and roles for claudin-family tight junction proteins. *IUBMB Life.* 2009; 61; 431-437.
- 14 Gori F, Thomas T, Hicok KC, Spelsberg TC, Riggs BL. Differentiation of human marrow stromal precursor cells: bone morphogenetic protein-2 increases OSF2/CBFA1, enhances osteoblast commitment, and inhibits late adipocyte maturation. *J Bone Miner Res.* 1999; 14:1522-1535.
- 15 Schultz Tj, Tseng Y-H. Emerging Role of Bone Morphogenetic Proteins in Adipogenesis and Energy Metabolism. *Cytokine Growth Factor Rev.* 2009; 20: 523-531.
- 16 Zamani N, Brown CW. Emerging Roles for the Transforming Growth Factor- Superfamily in Regulating Adiposity and Energy Expenditure. *Endocrine Reviews.* 2011; 32: 387-403.
- 17 Soon-Lee M, Lee H-J, Lee H-S, Kim Y. L-Carnitine stimulates lipolysis via induction of the lipolytic gene expression and suppression of the adipogenic gene expression in 3T3-L1 adipocytes. *Journal Medicinal Food.* 2007; 9: 468-473.



**Figure 6:** Upper pictures of a representative subject (age 57) illustrating placebo skin site at baseline (Fig 6a) and Chenopodium Quinoa Seed Extract skin site at baseline (Fig 6b). Lower pictures illustrating skin sites treated 28 days with placebo (Fig 6c) and Chenopodium Quinoa Seed Extract (Fig 6d). This subject reports a 45% decrease in eyelid roughness and a 29% decrease in eyelid folds after 28 days of treatment with Chenopodium Quinoa Seed Extract (Fig 6d) compared to baseline and visibly smoother and more lifted eyelids compared to placebo (Fig 6c).