

## Inhibitory Activities of Omega-3 Fatty Acids and Traditional African Remedies on Keloid Fibroblasts

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**Abstract:** Keloids develop when scar tissue responds to skin trauma with proliferative fibrous growths that extend beyond the boundaries of the original wound and progress for several months or years. Keloids most frequently occur in individuals of indigenous sub-Saharan African origin. The etiology for keloids is still unknown and treatment can be problematic as patients respond differently to various treatment modalities. Keloids have a high rate of recurrence following surgical excision. Some West African patients claim to have had successful outcomes with traditional African remedies—boa constrictor oil (BCO) and shea butter—leading the authors to investigate their effects on cultured fibroblasts. The effects of emulsions of BCO, fish oil, isolated omega-3 fatty acids, and shea butter were tested in comparison to triamcinolone regarding inhibition of cell growth in keloid and control fibroblast cultures. In a series of controlled studies, it was observed that fish oil and BCO were more effective than triamcinolone, and that cis-5, 8, 11, 14, 17-eicosapentaenoic acid was more effective than  $\gamma$ -linolenic acid. While cell counts in control cultures continuously decreased over a period of 5 days, cell counts in keloid cultures consistently declined between day 1 and day 3, and then increased between day 3 and day 5 for all tested reagents except for fish oil. These results suggest that oils rich in omega-3 fatty acids may be effective in reducing actively proliferating keloid fibroblasts. Additional studies are warranted to investigate whether oils rich in omega-3 fatty acids offer effective and affordable treatment for some keloid patients, especially in the developing world.

**K**eloids are a product of a dysregulated wound healing process, which may occur sporadically or they can be inherited. The tumor-like growths are often nodular in shape and always extend beyond the boundaries of the original wound margin (Figure 1). These lesions occur most commonly in individuals of darker pigmentation, with African and Asian populations being the most frequently affected. Keloids usually occur following trauma to the skin, inflammation, burns, surgery, but they may also arise from cutaneous viral and bacterial infections.<sup>1</sup>



**Figure 1.** Typical keloid scars on the chest, which extend over the border of the original wound and have been progressing for several years.

The processes that result in keloid scar formation are complex and involve the differential regulation of numerous cellular mechanisms. Studies have shown, for example, increased proliferation of keloid fibroblasts and differential protein expression compared to normal skin fibroblasts. These findings have led to several hypotheses for keloid formation in the literature.<sup>2</sup> Such hypotheses include abnormal regulation of growth factors and their receptors, hypoxia, reduced proteolytic degradation of the extracellular matrix, reduced apoptosis, and prolonged inflammatory and cytokine-mediated processes. There is also a genetic component to keloid formation, as keloids occur in an inheritable, as well as sporadic form.<sup>3</sup> However, the molecular trigger underlying keloid formation remains unknown.

Keloids, which can cause significant pain, pruritus, physical disfigurement, and contractures, present a therapeutic challenge. Several treatment options are available, which include treatment with corticosteroids such as triamcinolone acetonide either by injection or topical application,<sup>4,5</sup> low dose radiation,<sup>6</sup> pressure and silicone dressings, laser treatment,<sup>7</sup> cryotherapy, or surgical excision with or without adjunct therapies,<sup>8,9</sup> mostly intraleisional triamcinolone injection. However, treatment of keloids is still rather ineffective and it appears that available treatment modalities result in improvement rates of 70% or less.<sup>10</sup>

Traditional keloid remedies have been in use for generations. The Yoruba of South Western Nigeria, for example, use several methods to reduce the symptoms of keloids. Boa constrictor oil from the snake's visceral fat and ointments of shea butter (from nuts of the *Vitellaria paradoxa* tree), locally produced in Nigeria, are two

commonly used traditional medications. Some patients seen in the authors' clinics in Osogbo and Ibadan, Nigeria who have been using either shea butter or snake fat, reported observable remission of symptoms and swelling. In a number of cases the keloid scars have flattened out. Others present to the hospital after these traditionally used agents have failed.

Boa constrictor oil is rich in essential fatty acids,<sup>11,12</sup> which have been shown to have anti-inflammatory properties.<sup>13</sup> Shea butter is traditionally used as a skin care product due to its hydrating properties and its ability to soften scar tissues.

#### KEYPOINTS

- Treatment of keloids is still rather ineffective and it appears that available treatment modalities result in improvement rates of 70% or less
- The present study was designed to investigate the *in-vitro* effects of two fatty acid-containing substances traditionally used in Nigeria (BCO and shea butter) and omega-3 fatty acids

Indeed, fatty acid deficiencies in keloid tissues have been suggested as a cause for keloids in studies that have not received widespread attention among keloid researchers.<sup>11,13,14</sup> Lipids such as polyunsaturated fatty acids (PUFA) play a key role in cell membrane structure and are indispensable for the realization of anabolic events during tissue reconstruction.<sup>15</sup> Several studies suggest beneficial effects of polyunsaturated fatty acids. n-3 polyunsaturated fatty acids were shown to ameliorate symptoms of a range of immune-mediated conditions,<sup>16</sup> and local application of linoleic acid helped to reduce

scarring of burn wounds.<sup>11</sup> According to another study, keloids contain lower amounts of omega-6 fatty acids (linoleic acid [LA]),  $\gamma$ -linolenic acid (GLA), dihomo- $\gamma$ -linolenic acid (DGLA), omega-3 fatty acids ( $\alpha$ -linolenic acid [ALA]), and eicosapentaenoic acid (EPA) in combination with enhanced arachidonic acid (AA), which has pro-inflammatory properties.<sup>13</sup> Omega-3 fatty acids are polyunsaturated essential fatty acids that cannot be synthesized by humans and are produced by a variety of dietary sources, such as certain plants and fish. Omega-3 fatty acid levels are also high in oils of reptiles, specifically snakes.<sup>12</sup> Application of *Boa constrictor* oil (BCO) to keloid and control fibroblast cultures suggested that local application of fatty acids may inhibit fibroblast growth, thus exerting beneficial therapeutic effects.<sup>17</sup>

The present study was designed to investigate the *in vitro* effects of two fatty acid-containing substances traditionally used in Nigeria (BCO and shea butter) and omega-3 fatty acids. The inhibitory action of these traditional remedies and omega-3 fatty acids on keloid fibroblast growth were compared to that of triamcinolone acetonide (a corticosteroid most commonly used in the treatment of keloids). This study shows that omega-3 fatty acids inhibit fibroblast growth *in vitro*. These findings may lead to the development of an alternative treatment option in the management of keloids.

## Methods

**Cell culture.** Normal skin fibroblasts from a control subject and keloid fibroblasts from the proliferative outer zone of a keloid lesion were obtained from surplus tissues after medically indicated surgery. Subjects were consented with approval of the University of Connecticut Health Center Institutional Review Board (IRB). Cells were cultured in DMEM high glucose (Dulbecco's Modified Eagle Medium, [Invitrogen, Carlsbad, CA]) supplemented with penicillin/streptomycin, glutamine, and 10% fetal bovine serum (FBS). Cells were plated 24 hours prior to treatment either in 24-well plates (5500 cells/well) or 12-well plates (40,000 cells/well). Cells were treated 24 hours after plating (day 0). Cultures were terminated at days 1, 3, and 5. Day 1 is 1 day after the agents were added (baseline). Cells for each treatment group and time point were plated in triplicate and experiments were repeated twice.

The increasing concentrations (0.025%, 0.05%, 0.1%) of BCO and shea butter were compared to the same concentrations of mineral oil (control), fish oil, and triamcinolone acetonide (40 mg/mL) to determine the effects of

BCO and shea butter on fibroblast growth. *Boa constrictor* visceral fat and shea butter were purchased fresh at Igbona market in Osogbo, Nigeria and autoclaved. Mineral oil and soy oil (generic brand) were used as control substances. Fish oil (300 mg/mL omega-3, [Kirkland Brand, Costco, Kirkland, WA]) was used at concentrations of 0.0005%, 0.0025%, 0.005%, 0.025%, 0.05%, and 0.1% diluted in cell culture medium.  $\alpha$ -Linolenic acid and cis-5,8,11,14,17-Eicosapentaenoic acid were diluted in 200-proof ethanol and applied at final concentrations of 10, 25, and 50 g/mL (in 50 l/mL ethanol) using 50 l/mL ethanol as control.

All oils were sonicated at 1% concentration in cell culture medium using a Kontes Micro Ultrasonic Cell Disruptor. The vibrating probe generated fine emulsions that persisted throughout the cell culture period.

**Cell number determination.** Cells grown in 24-well plates were counted on days 1 (24 hours after addition of agents), 3, and 5. Cultures were trypsinized and counted using a hemacytometer. Cell viability was determined by trypan blue dye exclusion. Cell counts were performed in quadruplicates.

As a second method of cell number determination, dsDNA absorption of cells grown in 12-well plates at days 1, 3, and 5 was quantitated using Quant-iT PicoGreen dsDNA assays (Invitrogen) according to manufacturer's instructions. Three wells were plated for each time point and the experiment was performed in duplicate.

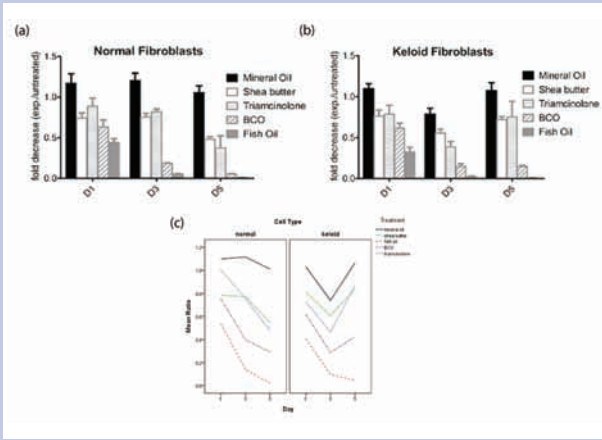
## Statistical Analysis

The general linear model was used to perform a factorial analysis of variance (ANOVA) to determine the main and interaction effects that four factors have on cell count ratios. The four factors were cell type (normal, keloid), concentration (0.1%, 0.05%, 0.025%), treatment (mineral oil, shea butter, fish oil, BCO, triamcinolone), and day (1, 3, 5). Cell count ratios, the outcome variable, were calculated by dividing the cell counts from five different

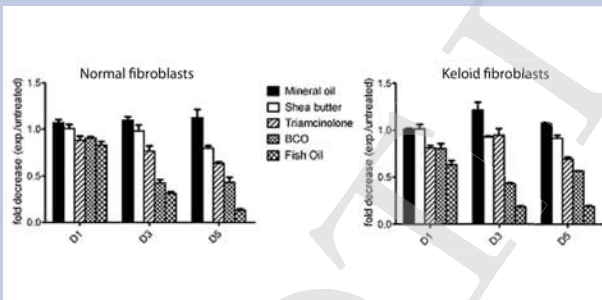
### KEYPOINTS

- While all tested agents resulted in significant cell growth arrest of normal and keloid fibroblasts compared to untreated or mineral oil treated cultures, fish oil was the most efficient, followed by BCO, triamcinolone, and lastly shea butter
- Omega-3 fatty acids are the likely mediators of growth inhibition, as fish oil, BCO, ALA, and EPA all had similar results regarding cell growth inhibition; EPA was the most efficient agent

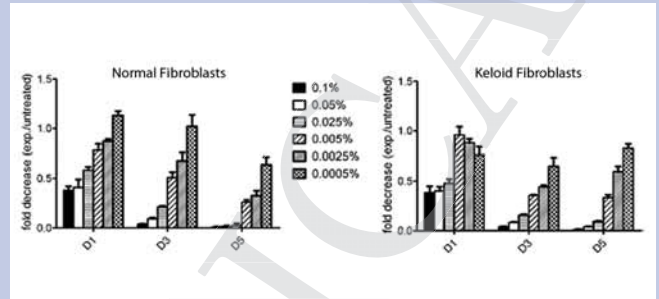




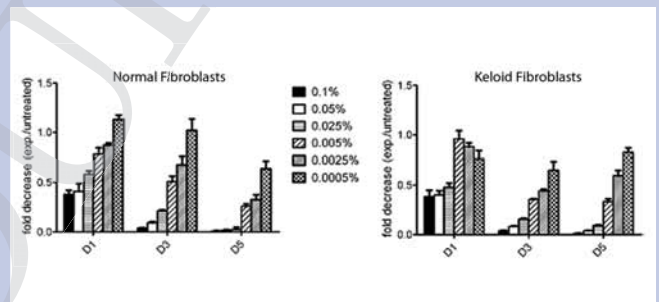
**Figure 2.** A) Fold difference of normal keloids. B) Keloid fibroblasts treated with different agents at concentrations of 0.1% compared to untreated groups by cell counting. Note that fish oil appears to be the most efficient treatment in control and keloid fibroblasts. C) The fold-differences of fibroblast counts in comparison to the control group suggest that keloid fibroblasts may develop some recalcitrance against treatments (Mean ratio = number of cell counts in experimental group/cell counts in control group).



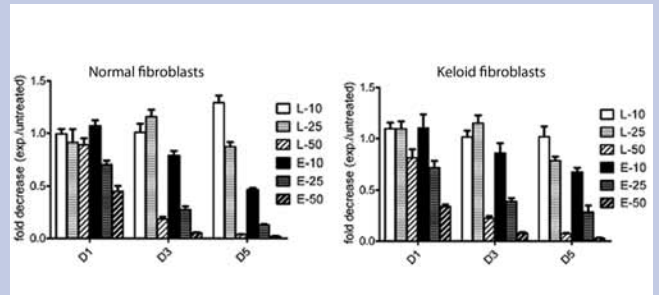
**Figure S1.** The fold differences of fibroblast numbers in comparison to the untreated control group at days 1, 3, and 5 after treatment was determined for each experimental group by DNA quantification with PicoGreen analysis in parallel cultures.



**Figure 3.** Fold decrease after treating normal and keloid fibroblasts with different concentrations of fish oil. There is no significant difference between keloid and control fibroblasts at high concentrations of fish oil (0.1, 0.05, and 0.025%), but at lower doses (0.005, 0.0025, 0.0005%) a dose-dependent effect on the growth of normal and keloid fibroblasts is seen (results by cell counting).



**Figure 4.** Comparison of fish oil to soy oil. Cell growth of normal and keloid fibroblasts after treatment with 0.025%, 0.05% and 0.1% of soy oil and fish oil emulsions shows that the inhibitory effect of fish oil is not caused by its diluent, soy oil.



**Figure 5.** Effects of linolenic acid (L) and eicosapentaenoic acid (E) on normal and keloid fibroblasts at doses of 10, 25, and 50 mg/mL. At lower doses, eicosapentaenoic acid (E) was more effective than linolenic acid (L). The efficiencies of all three doses differ significantly from each other. Linolenic acid ratio ( $\bar{x} = 0.81$ ) was significantly higher than the eicosapentaenoic acid ratio ( $\bar{x} = 0.47$ ) suggesting that eicosapentaenoic acid is most likely the active ingredient in fish oil.

**Table 1A.** Treatment of normal and keloid fibroblasts with different agents at 0.1% concentration by cell counting. The ratios represent fold difference in fibroblast numbers compared to untreated normal or keloid control groups.

Normal fibroblasts					
Day	Mineral oil	Shea butter	Triamcinolone	BCO	Fish oil
1	1.172 ± 0.27	0.743 ± 0.15 <sup>a</sup>	0.888 ± 0.24 <sup>a</sup>	0.634 ± 0.20 <sup>ac</sup>	0.442 ± 0.11 <sup>abc</sup>
3	1.206 ± 0.21	0.756 ± 0.10 <sup>a</sup>	0.820 ± 0.08 <sup>a</sup>	0.184 ± 0.02 <sup>abc</sup>	0.051 ± 0.02 <sup>abc</sup>
5	1.058 ± 0.19	0.480 ± 0.07 <sup>a</sup>	0.376 ± 0.36 <sup>a</sup>	0.054 ± 0.00 <sup>abc</sup>	0.009 ± 0.00 <sup>abc</sup>
Keloid fibroblasts					
1	1.095 ± 0.15	0.765 ± 0.17 <sup>a</sup>	0.786 ± 0.26 <sup>a</sup>	0.616 ± 0.14 <sup>a</sup>	0.326 ± 0.14 <sup>abcd</sup>
3	0.786 ± 0.17	0.556 ± 0.11	0.386 ± 0.16 <sup>a</sup>	0.151 ± 0.07 <sup>ab</sup>	0.024 ± 0.01 <sup>abc</sup>
5	1.073 ± 0.24	0.721 ± 0.09 <sup>a</sup>	0.753 ± 0.46 <sup>a</sup>	0.148 ± 0.03 <sup>abc</sup>	0.008 ± 0.00 <sup>abc</sup>

Data represent mean ± SD.

<sup>a</sup>Significant difference compared to mineral oil

<sup>b</sup>Significant difference compared to shea butter

<sup>c</sup>Significant difference compared to triamcinolone

<sup>d</sup>Significant difference compared to BCO

**Table 1B.** Treatment of normal and keloid fibroblasts with different agents at 0.05% concentration by cell counting. The ratios represent fold difference in fibroblast numbers compared to untreated normal or keloid control groups

Normal fibroblasts					
Day	Mineral oil	Shea butter	Triamcinolone	BCO	Fish oil
1	1.02 ± 0.34	0.76 ± 0.18 <sup>a</sup>	1.03 ± 0.15 <sup>b</sup>	0.81 ± 0.20	0.48 ± 0.17 <sup>abcd</sup>
3	1.08 ± 0.05	0.71 ± 0.25 <sup>a</sup>	0.70 ± 0.23 <sup>a</sup>	0.45 ± 0.10 <sup>ab</sup>	0.10 ± 0.02 <sup>abcd</sup>
5	1.06 ± 0.17	0.52 ± 0.12 <sup>a</sup>	0.49 ± 0.11 <sup>a</sup>	0.33 ± 0.17 <sup>a</sup>	0.01 ± 0.01 <sup>abcd</sup>
Keloid fibroblasts					
1	1.048 ± 0.13	0.812 ± 0.17 <sup>a</sup>	0.679 ± 0.22 <sup>a</sup>	0.665 ± 0.19 <sup>a</sup>	0.396 ± 0.13 <sup>abcd</sup>
3	0.738 ± 0.12	0.512 ± 0.16 <sup>a</sup>	0.480 ± 0.14 <sup>a</sup>	0.291 ± 0.07 <sup>ab</sup>	0.094 ± 0.02 <sup>abc</sup>
5	1.065 ± 0.18	0.895 ± 0.15	0.829 ± 0.28 <sup>a</sup>	0.408 ± 0.07 <sup>abc</sup>	0.036 ± 0.01 <sup>abcd</sup>

Data represent mean ± SD.

<sup>a</sup>Significant difference compared to mineral oil

<sup>b</sup>Significant difference compared to shea butter

<sup>c</sup>Significant difference compared to triamcinolone

<sup>d</sup>Significant difference compared to BCO

treatments by the average cell count from control cell cultures. There were six replicates for each factor combination resulting in 540 total observations. The Tukey Honestly Significance Difference (HSD) test was used as a post-hoc procedure for the pair-wise comparisons of the treatments. The data analysis was conducted with SPSS v17 software (IBM, Somers, NY).

## Results

To determine whether the traditional Nigerian keloid

remedies (BCO and shea butter) have any effect on the growth of keloid fibroblasts, a comparison was made regarding the effects of various concentrations (0.025%, 0.05%, 0.1%) of mineral oil, shea butter, BCO, fish oil, and triamcinolone acetone on the growth of fibroblasts derived from skin of a healthy control subject and the proliferative zone of a keloid tissue. Mineral oil served as the control. Fish oil was chosen as the comparator to BCO and shea butter because of its known content in omega-3 fatty acids and triamcinolone because it is a

**Table 1C.** Treatment of normal and keloid fibroblasts with different agents at 0.025% concentration by cell counting. The ratios represent fold difference in fibroblast numbers compared to untreated normal or keloid control groups.

Normal fibroblasts					
Day	Mineral oil	Shea butter	Triamcinolone	BCO	Fish oil
1	1.094 ± 0.31	0.841 ± 0.26	1.084 ± 0.20	0.802 ± 0.13	0.675 ± 0.19 <sup>ac</sup>
3	1.052 ± 0.19	0.842 ± 0.13	0.743 ± 0.42 <sup>a</sup>	0.553 ± 0.14 <sup>a</sup>	0.261 ± 0.18 <sup>abc</sup>
5	0.912 ± 0.16	0.636 ± 0.12	0.548 ± 0.23 <sup>a</sup>	0.479 ± 0.17 <sup>a</sup>	0.039 ± 0.04 <sup>abcd</sup>
Keloid fibroblasts					
1	0.944 ± 0.13	0.853 ± 0.08	0.710 ± 0.14	0.568 ± 0.08 <sup>ab</sup>	0.495 ± 0.14 <sup>ab</sup>
3	0.690 ± 0.33	0.748 ± 0.14	0.522 ± 0.11	0.416 ± 0.06 <sup>ab</sup>	0.165 ± 0.03 <sup>abc</sup>
5	1.050 ± 0.18	0.896 ± 0.21	1.011 ± 0.35	0.712 ± 0.12 <sup>ac</sup>	0.092 ± 0.01 <sup>abcd</sup>

Data represent mean ± SD.

<sup>a</sup>Significant difference compared to mineral oil

<sup>b</sup>Significant difference compared to shea butter

<sup>c</sup>Significant difference compared to triamcinolone

<sup>d</sup>Significant difference compared to BCO

pharmacological agent used in standard keloid treatment. The fold-differences of fibroblast numbers in comparison to the control group at days 1, 3, and 5 after treatment was determined for each experimental group by cell counting (Figure 2) and by DNA quantification (PicoGreen analysis; Figure S1) in parallel cultures. Results obtained by both methods were consistent. Cells in mineral oil continued to grow at a similar rate as untreated cells (no statistical difference;  $P = 0.69$ ), which suggests that the presence of the oil emulsion did not affect the fibroblast cultures. There was a significant two-way medium size effect (partial  $\eta^2 = 0.19$ ) for the interaction between cell type and day ( $P < 0.001$ ). The ratios within the normal fibroblasts for all four treatments (excluding the control mineral oil) showed a linear decrease by day, where day 3 was less than day 1 and day 5 was less than day 3, while the trend within the keloid fibroblasts was quadratic (decreased from day 1 to day 3, but increased from day 3 to day 5; Figure 2C). There was also a large main effect for treatment ( $P < 0.001$ ; partial  $\eta^2 = 0.73$ ), meaning the fold decrease in cell count differed significantly among the five treatments. While all tested agents resulted in significant cell growth arrest of normal and keloid fibroblasts compared to untreated or mineral oil treated cultures, fish oil was the most efficient (the mean fold decrease was:  $\bar{x} = 0.21$ ) followed by BCO ( $\bar{x} = 0.46$ ), triamcinolone ( $\bar{x} = 0.71$ ), and shea butter ( $\bar{x} = 0.73$ ). Each of the pair-wise comparisons was statistically significant (all  $P$  values  $< 0.001$ ) except between shea

butter and triamcinolone ( $P = 0.64$ ). There was a small main effect for concentration ( $P < 0.001$ ; partial  $\eta^2 = 0.07$ ) with the 0.1% concentration having the smallest ratio ( $\bar{x} = 0.57$ ), followed by 0.05% ( $\bar{x} = 0.62$ ), and then 0.025% ( $\bar{x} = 0.68$ ). Tables 1A–C demonstrate that while the inhibitory pattern of all three concentrations differed, the difference was negligible. In normal fibroblasts, the lowest cell counts were obtained at treatment day 5. Interestingly, at day 5 the keloid fibroblasts were more resistant to shea butter, BCO, and triamcinolone than at day 3. This pattern was seen in all wells of both experimental repeats indicating that keloid fibroblasts may develop some recalcitrance to these treatments. Fish oil treatment of cell cultures at those concentrations almost completely eliminated control and keloid fibroblasts after 5 days of treatment.

The effectiveness of lower doses of fish oil was examined since all three concentrations of fish oil appeared to severely inhibit fibroblast growth (Figure 3). Normal and keloid fibroblasts were treated with 0.005%, 0.0025%, and 0.0005% of fish oil emulsion in addition to the previous concentrations. A dose-dependent inhibitory effect of fish oil in both cell types at lower concentrations was seen (Figure 3). Even with the lowest dose of fish oil (0.0005%) treatment still resulted in significantly reduced fibroblast growth compared to the control cultures.

Next, the components of the fish oil extract that may contribute to the inhibitory effect on fibroblast growth were investigated. The omega-3 concentration of the

commercial fish oil extract was normalized by the addition of soy oil. To confirm that the effects from the fish oil extract were not caused by the soy oil, 0.025%, 0.05%, and 0.1% dilutions of soy oil treatment were compared to fish oil extract in normal and keloid fibroblast cultures (Figure 4). Fibroblast growth was significantly reduced after fish oil treatment suggesting that some active ingredients in fish oil may be responsible.

$\alpha$ -Linolenic acid (ALA) and cis-5,8,11,14,17-Eicosapentaenoic acid (EPA) are known to be the major beneficial omega-3 fatty acid components in fish oil. Therefore, the effect of 10, 25, and 50 mg/mL of ALA and EPA was tested on normal and keloid fibroblasts. There was a significant interaction between dose and fatty acid ( $P < 0.001$ ; partial  $\eta^2 = 0.33$ ). ALA was ineffective at 10 and 25 mg/mL, but effective at 50 mg/mL, while EPA showed a decrease in fibroblast numbers already at 10 mg/mL and was effective at 25 and 50 mg/mL (Figure 5). There was a large effect for dose ( $P < 0.001$ ; partial  $\eta^2 = 0.85$ ) and fatty acid ( $P < 0.001$ ; partial  $\eta^2 = 0.68$ ). All three levels of dose significantly differed from each other and the trend appeared to be linear with the 10 mg/mL having the highest ratio ( $\bar{x} = 0.95$ ) followed next by 25 mg/mL ( $\bar{x} = 0.71$ ), and then 50 mg/mL ( $\bar{x} = 0.27$ ). The ALA ( $\bar{x} = 0.81$ ) was significantly higher than EPA ( $\bar{x} = 0.47$ ). This experiment shows that EPA is most likely the active ingredient in fish oil, and possibly in BCO.

## Discussion

Keloids are erythematous nodules, which are frequently symptomatic with most patients reporting tenderness and pruritus. Keloids affect millions of patients worldwide and aside from pain and irritation, keloids can also have severe psychosocial and socioeconomic consequences. Patients often have concerns about their appearance and feel stigmatized by their environment. The pathology of keloids is not well understood though there are several theories that have been postulated. One interesting and little observed hypothesis is the fatty acid theory,<sup>11,13</sup> which has been postulated after observing lower fatty acid contents in keloid and hypertrophic tissues.

Polyunsaturated fatty acids are contained in oils, such as fish oil, BCO and certain plant oils. Oil from snakes has profoundly negative implications in Western cultures. The mere mention of the term "snake oil" in the United States immediately brings thoughts of the charlatan or quack. Yet, snakes have been part of Far Eastern and African medicine for centuries. Paradoxically, the snake adorns the rod of Aesculapius, a symbol of the

Western medical profession. In Nigeria (the country with the largest black population and where keloids are a widespread medical issue), several traditional medical keloid remedies are commonly used. The most common treatments are BCO and shea butter. Some patients have presented to the authors' clinics in Nigeria with flattened and non-symptomatic keloids. Many other patients have tried these remedies with no remission of symptoms. This is not surprising as it is well known that no single form of medical treatment works for every patient, and success rates for the most common treatments are 70% or less.<sup>10</sup>

Boa constrictor oil and fish oil are rich in omega-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA).<sup>12</sup> Therefore, the effect of BCO on fibroblast cell growth was compared to fish oil and their active ingredients, omega-3 fatty acids. A certain reduction of fibroblast cell counts after treatment with BCO had been shown previously.<sup>17</sup> The fat obtained from snake (usually from the skin and viscera) is used in local medicines for the treatment of burns and inflammatory conditions and has shown some efficacy in laboratory experiments.<sup>18</sup> Shea butter is extracted by grinding and boiling the fruit of the shea tree and is used as moisturizer in cosmetics and in the food industry as substitute for cocoa butter. Shea butter is alleged to have anti-inflammatory properties and contains regionally varying proportions of saturated and unsaturated fatty acids.<sup>19</sup> In traditional Nigerian medicine, shea butter is used for several ailments including ulcers, scabies, nasal stuffiness, and other conditions.<sup>20</sup> Even today, snake fat and shea butter are widely used, especially in rural populations, because they are cheap and readily available and considered by some keloid patients as the best therapy for their skin lesions. BCO (often raw fat) and shea butter are topically applied daily over a prolonged period of time until symptoms subside. Shea butter melts easily on application to the skin and is applied to the keloid either alone or in combination with a body lotion.

The authors initiated this study to compare the efficacy of BCO and shea butter because of their frequent use in our Nigerian study population. Compared to control fibroblast cultures, which were either untreated (data not shown) or treated with mineral or soy oil, both remedies showed reduction in fibroblast growth. However, the inhibitory effect of BCO at all tested concentrations was stronger than that of shea butter. Under our experimental conditions BCO outperformed triamcinolone acetate (TA), the most widely used corticosteroid for



**KEYPOINTS**

- The mechanisms for growth inhibition of omega-3 fatty acids are not well understood
- Further studies are warranted to test whether EPA-containing oils could be part of a treatment regime for some keloid patients

intralesional keloid injection. Triamcinolone has been shown to inhibit the growth kinetics of fibroblasts in culture.<sup>21</sup> However, BCO was less efficient than fish oil with a defined content of omega-3 fatty acids. The authors determined that the oil emulsions act in a dose dependent manner on cultured cells by testing a wide range of concentrations and the authors speculated that the active ingredient for fibroblast cell growth inhibition are omega-3 fatty acids. The omega-3 fatty acid content in BCO was not determined, and therefore, the authors will not make any claims to the efficiency of omega-3 fatty acids in the oil; however, snake oils have been shown to be enriched in EPA.<sup>12</sup> When the cultures were treated with  $\alpha$ -linolenic acid (ALA) and eicosapentaenoic acid (EPA), the authors showed that EPA had a significant inhibitory effect on fibroblast growth curves, while ALA had a modest effect. The ALA and EPA concentrations that were used led to dose-dependent inhibition and were within the dosage range used in previous studies to determine omega-3 fatty acid effects on wound healing and cell growth arrest.<sup>22-24</sup>

While the full mechanism for omega-3 fatty acid inhibition of cell growth is not known, there are a number of (mostly cancer cell) studies where fish oil or omega-3 fatty acids have been implicated in down-regulating anti-apoptotic signals and in blocking pro-inflammatory signals that lead to increased cell proliferation.<sup>25,26</sup> It is therefore likely that omega-3 fatty acids reduce fibroblast cell growth by a number of mechanisms including reduced expression of cytokines or other effects on gene regulation.<sup>14</sup>

Omega-3 (n-3) polyunsaturated fatty acids (PUFA) and n-6 polyunsaturated fatty acids compete for metabolism to eicosanoids, and it has been shown that dietary n-3 polyunsaturated fatty acid supplementation is associated with increased incorporation into skin and with reduced basal and UVB-induced skin prostaglandin E2 (PGE2) levels.<sup>27,28</sup> Omega-3 polyunsaturated fatty acid supplementation has also been shown to reduce secretion of pro-inflammatory cytokines, tumor necrosis factor (TNF- $\alpha$ ), and interleukin (IL-1 $\beta$ ) in circulating monocytes.<sup>16,29,30</sup> Administration of PUFAs has been reported to be bene-

ficial in a range of inflammatory conditions.<sup>16</sup> PUFAs reduce, for example, the effect of TNF- $\alpha$ , which suggests a wider range of anti-inflammatory properties for these agents<sup>31</sup> and may induce PGE3, which in turn may subdue cellular response to mitogenic and inflammatory stimuli.<sup>32</sup> Administration of PUFAs has been reported to be beneficial in a range of immune-mediated conditions<sup>16</sup> and local application of linoleic acid to burn wounds has been shown to improve healing by decreasing hypertrophic scarring.<sup>11</sup>

Louw<sup>13</sup> observed decreased levels of omega-6 fatty acids: linoleic acid (LA),  $\gamma$ -linolenic acid (GLA), dihomo-g-linolenic acid (DGLA), omega-3 fatty acids ( $\alpha$ -linolenic acid [ALA] and eicosapentaenoic acid [EPA]) in combination with enhanced arachidonic acid (AA) levels in keloids.<sup>13</sup> This is in agreement with a previous work by Shakespeare and Strange,<sup>11</sup> who found a significantly lower level of essential fatty acids (Linoleic acids C 18:2) in phospholipids isolated from swabs of hypertrophic scars.

Keloid fibroblasts showed refractory behavior towards treatment in our comparative cell culture experiments. At day 5 of treatment the cell numbers increased compared to control fibroblasts suggesting that they are less responsive to the growth-inhibiting effects of the treatment agents. It is possible that this behavior is due to keloid-specific genetic regulation of PUFA metabolism or a lack of responsiveness to anti-proliferative signals. Higher concentrations of anti-mitotic stimuli may be required to inhibit the growth of keloid fibroblasts.

**Conclusion**

The present study found that traditional African keloid remedies, shea butter, and BCO, are effective in inhibiting growth of normal and keloid fibroblasts in culture. Omega-3 fatty acids are the likely mediators of growth inhibition, as fish oil, BCO, ALA, and EPA all had similar results regarding cell growth inhibition; EPA was the most efficient agent. The mechanisms for growth inhibition of omega-3 fatty acids are not well understood. Further studies are warranted to test whether EPA-containing oils could be part of a treatment regime for some keloid patients. Efficacy tests should be performed because some patients are concerned about side effects of corticosteroids or other cytotoxic drugs for keloid treatment, and many patients, especially in developing countries, cannot afford such treatment.

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