LAMINARIA DIGITATA EXTRACT INFLUENCES GENE EXPRESSION RELATING TO ADIPOGENESIS AND LIPOLYSIS AND THUS MAY HAVE USE AS AN ACTIVE FOR CELLULITE

H.E. Knagas; D.G. Kern; R. Gopaul
'Center for Anti-Aging Research, Nu Skin Enterprises, Inc., Provo, Utah, United States

BACKGROUND

Excess adipose tissue, specifically cellulite, can be located anywhere on the body containing subcutaneous fat and can be more pronounced in some areas of the body than others. It is most commonly seen on the upper outer thigh (Fig. 1) and the posterior thighs and buttocks, but can also be seen on the breasts and upper arms. In the medical literature, cellulite is known as adipoedematosis, dermatohypertrophic deformans, status protrusus cutis, etc. Cellulite is perceived as an uneven, bumpy skin texture seen especially with side lighting of the affected area. It has been described as an “orange peel” or “cottage cheese” skin appearance. This appearance is due to herniation of subcutaneous fat into the reticular and papillary dermis and can be documented via ultrasound as low-density regions among the denser dermal tissue1. Clinically, the severity of cellulite or the effectiveness of cellulite therapies is documented through the number and degree of these subcutaneous fat projections (Fig. 2).

The complete etiology of cellulite is unclear. Current theories revolve around genetic predisposition1, vascular insufficiency, changes in lipid metabolism, and structural changes in the extracellular matrix (ECM) of the skin. Two key processes responsible for fat deposition are adipogenesis, the differentiation of preadipocytes into adipocytes, and lipolysis, the breakdown of lipids via hydrolysis of triglycerides into free fatty acids for use in oxidative phosphorylation and energy production. An increase in adipogenesis and decrease in lipolysis can lead to localized fat deposition in certain areas of the body, potentially resulting in cellulite. Localized fat deposition may also cause the extracellular matrix (ECM) to be compromised, making the skin appear loose and wrinkled in some individuals. In this study, Laminaria digitata (L. digitata) extract was evaluated for its effects on lipolysis and adipogenesis and its use as an anti-cellulite agent. Essentially, body fat was studied to examine gene expression changes that may contribute to improved lipid metabolism and protection of the extracellular matrix in the skin.

OBJECTIVE

Investigate the ability of an extract of the seaweed, Laminaria digitata, to influence lipid metabolism in the skin and protect the extracellular matrix.

METHODS & MATERIALS

In this study, two concentrations of Laminaria digitata (L. digitata) extract were prepared in water (0.1% and 0.5%) and tested in vitro using two human cell systems. Human full-thickness 3D-epidermal skin equivalents (FTEE, MatTek, Ashland, MA) and primary human adipocytes (ZenBio, Research Triangle Park, NC) isolated from healthy, normal subcutaneous adipose tissue obtained from elective surgery were used as models.

FTEE CULTURES

100 μl of the test article was applied to each culture and incubated for 24 hours. Following incubation, in preparation for gene expression analysis, the cultures were thoroughly washed with sterile phosphate buffered saline (PBS) to remove test materials and placed in RNA later solution for two hours at room temperature followed by storage at -4°C until assayed.

PRIMARY NORMAL ADIPOCYTES

 Cultures were allowed to acclimate for five to seven days at 37°C, 5% CO2 in a humidified incubator prior to assay. The test article was diluted to final assay concentration in Lipo2/3 Assay Buffer. Adipocyte growth medium was replaced with adipocyte maintenance medium (AM-M) containing the test article at the desired final concentration and incubated for 24 hours at 37°C, 5% CO2 in a humidified incubator.

QPCR ANALYSIS

Custom Taqman Low Density Array cards (TLDA) were created using Life Technologies (Foster City, CA) validated gene expression assays. Each TLDA card contained 376 skin-relevant target genes selected from the published literature. In addition, five common endogenous control genes (GUSB, HBPRT, HMBS, GAPDH, and 18S) were included. One microgram of total RNA from each tissue sample was converted into cDNA using High Capacity cDNA Reverse Transcription kit from Life Technologies. An Applied Biosystems 7900HT Fast instrument was used for amplification and fluorescence detection.

STATISTICS

Data analysis for qPCR was carried out according to RQ analysis methods using RQ Manager and Stahlf ner (v3.1) software programs. Expression levels were determined based on relative quantification analysis, t-test with Benjamin and Hochberg false discovery rate correction (p value equal or less than 0.05) with a cycle threshold of less than 35.

RESULTS

Overall, using a 1.5-fold expression change threshold, in the adipocyte model, 22 genes were down regulated and 49 were up regulated and in the FTEE model, 54 were down regulated and 103 were up regulated. Compared to an untreated control, L. digitata extract regulated genes related to activation of lipolysis and reduction in adipogenesis on adipocyte cells, for example ASIP, PDESA, FFAR, and ADRP (Table 1). On the full-thickness epidermal equivalent model, genes known to improve the integrity of the extracellular matrix were favorably regulated, for example COLIA1, CTGF, DSG2, and TIMP1 (Table 2). The findings from this study suggest a possible value for L. digitata extract in improving lipid metabolism and protecting the extracellular matrix from degradation.

DISCUSSION

The theory that has received the most medical support contends that cellulite is an inflammatory process resulting from the breakdown of the collagen in the dermis, such that subcutaneous fat herniations into the dermis can be seen with ultrasound and skin texture changed, in extreme cases giving the typical appearance of snow.

The onset of cellulitis with puberty and menopause has caused some researchers to evaluate the hormonal changes necessary for the development of the endometrium2, specifically the secretion of collagenases (collagenase-1, MMP-1) and gelatinases (gelatinase A, MMP-2) as causative in the production of cellulite3. The endometrial glandular and stromal cells release these enzymes to allow menstrual bleeding to occur. Collagenases cleave the triple helical domain of fibril collagen chains at a neutral pH and are secreted just prior to menstruation. The secretion of endometrial collagenase to initiate menstruation also provides for collagen breakdown in the dermis4. This might also help to explain why cellulite is seen following parturition changes, as well as why it occurs to a greater extent in women. Thus, the hypothesis would be that the fluctuating hormone levels during menstruation initiate the events for cellulite formation in regions enriched with subcutaneous adipocytes in the body. The cascading events with concomitant production of enzymes responsible for degrading the ECM then play a role in disintegration of the dermal ECM and ensuing inflammation.

CONCLUSION

Our data suggest that a comprehensive cellulite treatment should reduce inflammation, address lipid metabolism, and protect the extracellular matrix.

TABLE 1 Lipid metabolism-related gene expression changes in the adipocyte model

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Name</th>
<th>Function</th>
<th>Log2 Fold Change</th>
<th>1. digitata Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>adipose differentiation-related protein</td>
<td>promotes conversion of pre-adipocytes to adipocytes</td>
<td>-1.936</td>
<td>-1.936</td>
</tr>
<tr>
<td>DETA1</td>
<td>DETA1</td>
<td>down regulates adipogenesis</td>
<td>-1.891</td>
<td>-1.891</td>
</tr>
<tr>
<td>MGAT1</td>
<td>MGAT1</td>
<td>increases triglyceride levels</td>
<td>1.074</td>
<td>1.074</td>
</tr>
<tr>
<td>FGF21</td>
<td>FGF21</td>
<td>regulates lipid degradation</td>
<td>-0.525</td>
<td>-0.525</td>
</tr>
</tbody>
</table>

TABLE 2 Extracellular matrix-related gene expression changes in the full-thickness epidermal equivalent (FTEE) model

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Name</th>
<th>Function</th>
<th>Log2 Fold Change</th>
<th>1. digitata Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL1A1</td>
<td>COL1A1</td>
<td>increases skin elasticity</td>
<td>1.074</td>
<td>1.074</td>
</tr>
<tr>
<td>COL2A1</td>
<td>COL2A1</td>
<td>increases collagen</td>
<td>1.074</td>
<td>1.074</td>
</tr>
<tr>
<td>COL3A1</td>
<td>COL3A1</td>
<td>increases elastin</td>
<td>1.074</td>
<td>1.074</td>
</tr>
<tr>
<td>COL4A1</td>
<td>COL4A1</td>
<td>increases laminin</td>
<td>1.074</td>
<td>1.074</td>
</tr>
</tbody>
</table>

REFERENCES