**L. DIGITATA INFLUENCES THE EXPRESSION OF GENES RELATED TO VASCULATURE AND MAY THEREFORE BE BENEFICIAL FOR IMPROVING THE APPEARANCE OF CELLULITE**

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**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 thighs 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 adipose edematosis, dermopanniculitis deformans, statura primitiva 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 herniations of subcutaneous fat into the reticular and papillary dermis and can be documented via ultrasound as low-density regions among the denser dermal tissues.

Clinically, the severity of cellulite or the effectiveness of various cellulite therapies is documented through the number and degree of these subcutaneous fat projections (Figure I).

The complete etiology of cellulite is unclear. Current theories revolve around genetic predisposition, structural changes in the extracellular matrix (ECM) of the skin, changes in lipid metabolism, and contribution from vascular insufficiency. Vascular constriction often accompanies the formation of cellulite, but it is not known whether results from increasing adipocyte size is or causative. Nevertheless, vascular constriction results in reduced blood flow, reducing nutrient supply to the upper areas of skin, weakening the skin's connective tissues, and possibly contributing to the dimpling effect seen in persons with cellulite. In this study, we choose to examine gene expression changes associated with the vasculature that may contribute to changes in fluid movement in the skin.

**OBJECTIVE**

Investigate the ability of an extract of the seaweed Laminaria digitata to influence vascular markers in the skin.

**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 material and placed in RNA later solution for 2 hours at room temperature followed by storage at -80°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 (AMM-3) 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 576 skin-relevant target genes selected from the published literature. In addition, five common endogenous control genes (GUSB, HPRT1, HBB, GAPDH, and 18S) were included. One micromolar 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 IT instrument was used for amplification and fluorescence detection.

**STATISTICS**

Data analysis for qPCR was carried out according to ROX analysis methods using ROX Manager and StatMiner (KS) software programs. Expression levels were determined based on relative quantitation 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. In the FTEE model 54 genes were down regulated and 103 were up regulated. Compared to untreated control, L. digitata extract up regulated genes related to vaso-dilation on epidermal skin equivalents and adipocytes. Compared to untreated control, L. digitata extract also down regulated genes related to vascostimulation on adipocytes. Genes that were up regulated include HIF1A, VEGFA, and HP. Genes that were down regulated include AGTR1 and ADRAR.

The findings from this study suggest a possible value for L. digitata extract in improving the appearance of cellulite by affecting gene expression changes that may have an impact in fluid movement in the skin.

**DISCUSSION**

The theory that has received the most medical support contends that cellulite is an inflammatory response resulting from the breakdown of the collagen in the dermis, causing subcutaneous fat herniations that can be seen using ultrasound.

The onset of cellulite with puberty and menstruation has caused some researchers to evaluate whether hormonal changes necessary for loosening of the endosome contribute to the formation of cellulite 
y, specifically the secretion of collagenases (collagenase-1, MMP-1) and gellastinases (gelatinase A, MMP-2), as causative in the production of cellulite. The endometrial glandular and stromal cells secrete these enzymes to allow menstrual bleeding to occur. Collagenases cleave the triple helical domain of fibrillar collagens at a neutral pH and are secreted just prior to menstruation. The secretion of endometrial collagenases to initiate menstruation also provides for collagen breakdown in the dermis. This might also help to explain why cellulite is seen following pubertal 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 concurrent production of enzymes responsible for degrading the ECM then play a role in disintegration of the dermal ECM and ensuing inflammation.

Furthermore, gelastinase B is produced by stromal cells or mast cells during the late proliferative endometrial phase and just after ovulation. Gelastinase B is associated with an influx of polymorphonuclear leukocytes, macrophages, and eosinophils, which also contribute to inflammation. A marker for inflammation is the synthesis of dermal glycosaminoglycans that enhance water binding, further worsening the appearance of the cellulite through swelling. The presence of these glycosaminoglycans has been observed on ultrasound as low-density echoes at the lower dermal/subcutaneous junction. Similar events may occur in the skin, causing the changes associated with cellulite.

With repeated cyclical collagenase production, more and more dermal collagen is destroyed, accounting for the worsening of cellulite seen with age. Eventually, enough collagen is destroyed to weaken the reticular and papillary dermis and allow subcutaneous fat to herniate between the structural fibrous septa found in female fat (more so than in males; female subcutaneous fat is requested into discrete pockets by the presence of septa). Obviously, if more subcutaneous fat is present, more pronounced herniation will occur, moving the skin upward while the septic hold areas of the skin in place. Deposition of the dermal vasculature, particularly constriction of or loss of the capillary network, also contributes to the process. As a result, excess fluid is retained within the dermal and subcutaneous tissues, limiting the removal of tissue-degrading enzymes and signal and choking the supply of oxygen-supporting oxidative respiration in favor of energy storage in the form of additional lipid deposition. This loss of the capillary network is thought to be due to engorged fat cells clumping together and inhibiting venous return. Using cultured adipocytes and full-thickness epidermal equivalent models within the initial treatment of 24 hours with L. digitata extract, we were able to detect a change in gene expression for genes relating to vascular control and circulation.

**CONCLUSION**

Our data suggest that a comprehensive cellulite treatment should address fluid movement in addition to enzymatic breakdown of the ECM, inflammation, and lipid metabolism.

**TABLE 1. Vascular-related gene expression changes in the adipocyte model**

<table>
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<tr>
<th>Gene ID</th>
<th>Name</th>
<th>Function</th>
<th>Stag</th>
<th>Log2 Expression</th>
<th>L. digitata Extract</th>
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**TABLE 2. Vascular-related gene expression changes in the full-thickness equivalent model (FTEE) model**

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**REFERENCES**