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 adisposis edematosa, dermopanniculosis deformans, status protrusus cutis, etc[1]. 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 tissue[2]. 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 1).

The complete etiology of cellulite is unclear. Current theories revolve around genetic predisposition[3], structural changes in the extracellular matrix (ECM) of the skin, changes in lipid metabolism, and a contribution from vascular insufficiency. Vasoconstriction often accompanies the formation of cellulite, but it is not known whether this results from increasing adipocyte size or is causative. Nevertheless, vasoconstriction 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 (1.0% 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 RNAlater solution for 2 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% CO_2 in a humidified incubator prior to assay. The test article was diluted to final assay concentration in LIP02/3 Assay Buffer. Adipocyte growth medium was replaced with adipocyte maintenance medium (AM-1) containing the test article at the desired final concentration and incubated for 24 hours at 37°C, 5% CO_2 in a humidified incubator.

QPCR ANALYSIS

Custom Taqman Low Density Array cards (TLDAs) 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, HPRT, 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 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 StatMiner (v3.1) software programs. Expression levels were determined based on relative quantification analysis, t-test with Benjamini 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 vasodilation on epidermal skin equivalents and adipocytes. Compared to untreated control, *L. digitata* extract also down regulated genes related to vasoconstriction on adipocytes. Genes that were up regulated include HIF1A, VEGFA, and HP. Genes that were down regulated include AGTR1 and ADRA1D. 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 process 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 sloughing of the endometrium contribute to the formation of cellulite[4], specifically the secretion of collagenases (collagenase-1, MMP-1) and gelatinases (gelatinase A, MMP-2), as causative in the production of cellulite[5]. 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 collagenase to initiate menstruation also provides for collagen breakdown in the dermis[6]. 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 concomitant production of enzymes responsible for degrading the ECM then play a role in disintegration of the dermal ECM and ensuing inflammation.

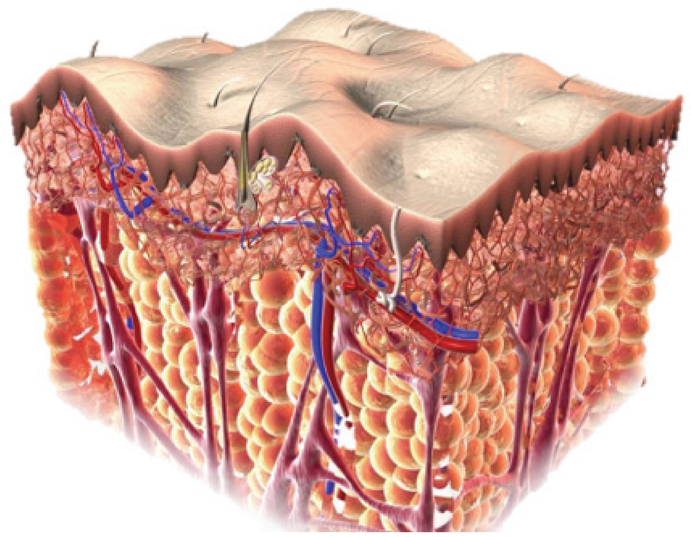

FIGURE 1. 3D representation of skin exhibiting a cellulite morphology.

Furthermore, gelatinase B is produced by stromal cells or mast cells during the late proliferative endometrial phase and just after ovulation. Gelatinase B is associated with an influx of polymorphonuclear leukocytes, macrophages, and eosinophils, which also contribute to inflammation[7]. A marker for this 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[8]. 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 sequestered into discrete pockets by the presence of septa). Obviously, if more subcutaneous fat is present, more pronounced herniation can occur, moving the skin upward while the septae hold areas of the skin in place. Deterioration of the dermal vasculature, particularly constriction of or loss of the capillary network, also contributes to the process[9]. As a result, excess fluid is retained within the dermal and subcutaneous tissues[10], limiting the removal of tissue-degrading enzymes and signals 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[11]. 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.

Gene ID	Name	Function	Reg	*L. digitata* Extract	
				1%	0.5%
AGTR1	angiotensin II receptor, type 1 or AT1 receptor	blocks angiogenesis	DOWN	2.194206	2.818034
ADRA1D	alpha-1D adrenergic receptor (α1D adrenoreceptor),	vasoconstriction	DOWN	1.923633	2.431518
HP	haptoglobin	involved in arterial reconstruction (breakdown of gelatin)/ stimulates angiogenesis	UP	2.630214	3.991538
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor	activates transcription of NOS, which promotes angiogenesis and vasodilation	UP	1.561311	1.660172

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

Gene ID	Name	Function	Reg	*L. digitata* Extract	
				1%	0.5%
ADRA1D	alpha-1D adrenergic receptor (α1D adrenoreceptor),	vasoconstriction	DOWN	4.412065	2.951799
VEGFA	vascular endothelial growth factor A	angiogenesis	UP	2.44878	2.271423
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor	activates transcription of NOS, which promotes angiogenesis and vasodilation	UP	1.801934	1.742663
HP	haptoglobin	involved in arterial reconstruction	UP		3.017439

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

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