LAMINARIA DIGITATA EXTRACT INFLUENCES GENE EXPRESSION RELATING TO ADIPOGENESIS AND LIPOLYSIS AND THUS MAY HAVE USE AS AN ACTIVE FOR 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 (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 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 (Fig. 2).

The complete etiology of cellulite is unclear. Current theories revolve around genetic predisposition[3], 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 hydroxylation 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 subsequent influences on the appearance of body skin. In this study, we choose 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 (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 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% 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 accordingly 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 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, PDE5A, KFL6, 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 COL4A1, 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 is changed, in extreme cases giving the typical appearance of snow.

The onset of cellulite with puberty and menstruation has caused some researchers to evaluate the hormonal changes necessary for sloughing of the endometrium[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.

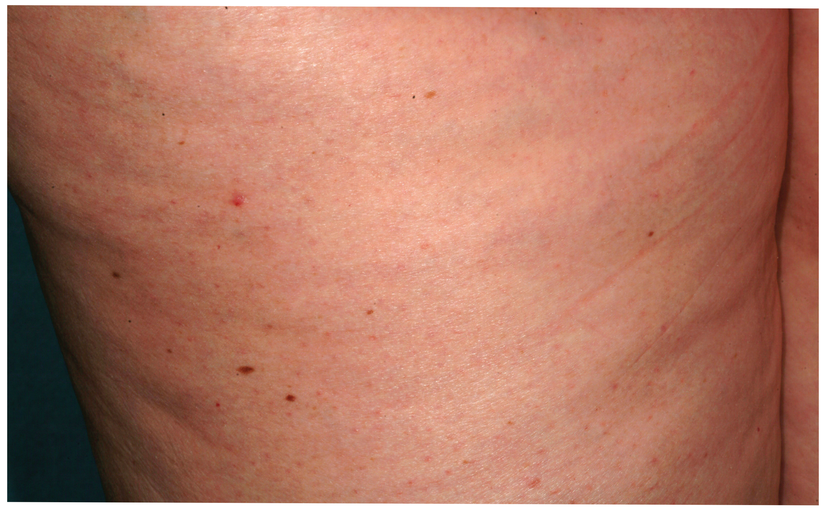

FIGURE 1. Typical appearance of cellulite on the upper outer thigh.

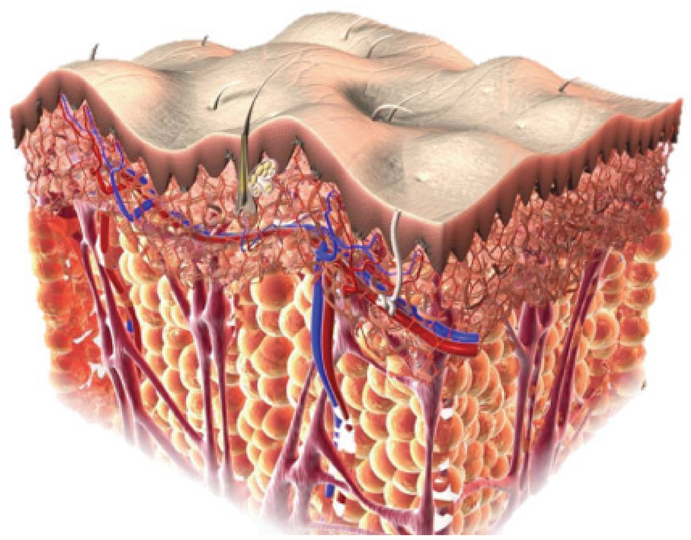

FIGURE 2. 3D representation of skin exhibiting a cellulite morphology.

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 catabolic signals and choking the supply of oxygen-supported respiration in favor of energy storage by additional lipid deposition. The compromising loss of an efficient capillary network with inhibited venous return[11] further enhances lipid deposition and ECM destruction.

In this study, we investigated gene changes that would help to mitigate these hormonal-induced changes and report benefits in terms of gene regulation for *L. Digitata* extract. Additional clinical studies using products formulated with this extract have shown benefits in the clinical appearance of cellulite.

CONCLUSION

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

Gene ID	Name	Function	Reg	*L. digitata* Extract	
				1%	0.5%
ASIP	agouti signaling protein	increases adiposity	DOWN	11.14134	10.35538
PDE5A	phosphodiesterase	inhibits lipolysis	DOWN	2.081708	1.850289
KLF6	Krüppel-like factor 6	positive regulator of adipogenesis through PPARγ	DOWN	1.903341	1.511707
ADRP	adipose differentiation-related protein	localizes in lipid droplets; plays a role in the management of neutral lipid stores, both in deposition and mobilization.	UP		1.548357

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

Gene ID	Name	Function	Reg	*L. digitata* Extract	
				1%	0.5%
COL4A1	collage type IV alpha 1	increases skin elasticity	UP	1.680723	1.5588
CTGF	connective tissue growth factor	increases collagen	UP	2.392926	2.304992
DSG2	desmoglein 2	cell adhesion	UP	1.557	
TIMP1	tissue inhibitor of matrix metalloproteinases 1	degrades MMP1	UP	1.645369	1.650898

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

REFERENCES

1. Dahl PR, Salla MJ, Winkelmann RK. Localized involutional lipoatrophy: a clinicopathologic study of 16 patients. J Am Acad Dermatol 1996; 35:523-8.
2. Salter DC, Hanley M, Tynan A, McCook JP. In-vivo high definition ultrasound studies of subdermal fat lobules associated with cellulite. J Invest Dermatol 1990;29:272-4.
3. Emanuele E, Bertona M, Geroldi D. A multilocus candidate approach identifies ACE and HIF1A as susceptibility genes for cellulite. J Eur Acad Dermatol Venereol 2012;24(8):930-5.
4. Marbaix E, Kokorine I, Henriet P, Donnez J, Courtoy PJ, Eeckhout Y. The expression of interstitial collagenase in human endometrium is controlled by progesterone and by oestadiol and is related to menstruation. Biochem J 1995;305:1027-30.
5. Singer CF, Marbaix E Lemoine P, Courtoy PJ, Eeckhout Y. Local cytokines induce differential expression of matrix metalloproteinases but not their tissue inhibitors in human endometrial fibroblasts. Eur J Biochem 1999;259(1-2):40-5.
6. Marbaix E, Kokorine I, Donnez J, Eeckhout Y, Courtoy PJ. Regulation and restricted expression of interstitial collagenase suggest a pivotal role in the initiation of menstruation. Hum Reprod 1996, 11 Supp 2:134-43.
7. Jeziorska M, Nagasae H, Salamonsen LA, Woolley DE. Immunolocalization of the matrix metalloproteinases gelatinase B and stromelysin 1 in human endometrium throughout the menstrual cycle. J Reprod Fertil 1996 107(1):43-51.
8. Lotti T, Ghersetich MD, Grappone C, Dini G. Proteoglycans in so-called cellulite. Int J Dermatol 1990;29:272-4.
9. Smith WF. Cellulite treatments: snake oil or skin science. Cosmet Toilet 1995;110:61-70.
10. Curri SB. Cellulite and fatty tissue microcirculation. Cosmet Toilet 1993;108:51-8.
11. Curri SB, Bombardelli E. Local lipodystrophy and districtual microcirculation. Cosmet Toilet 1994;109:51-65.