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Anti-Cellulite actives, dream or reality?

ABSTRACT

Cellulite becomes more and more of a problem in a world where a growing percentage of the population is suffering from overweight. Overweight due to excessive food uptake often goes hand in hand with the development of surplus subcutaneous fatty tissue. Although a calorie restricted diet and an exercise regime is effective in achieving a total weight loss there is often a less immediate influence on the fatty content of the subcutaneous tissue and the superficial appearance of the skin. The combination of a diet and exercising with the application of cosmetic products can work efficiently against the unpleasant appearance of cellulite or "orange-peel" skin .

There are basically two pathways which can be targeted to achieve cellulite reduction: the inhibition of lipogenesis to prevent fat storage in the adipocytes and lipolysis, the active breakdown of fatty tissue under the skin. Both processes support each other in a complimentary way and can provide an extra cosmetic benefit when applied as a topical treatment on the skin together with an appropriate diet and exercise regime.

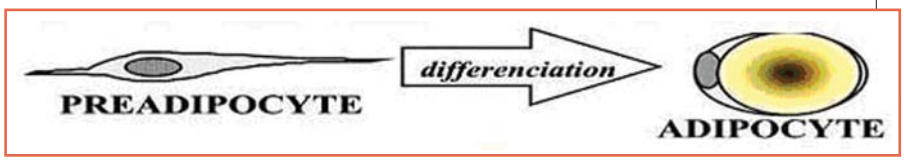


Figure 1

INTRODUCTION

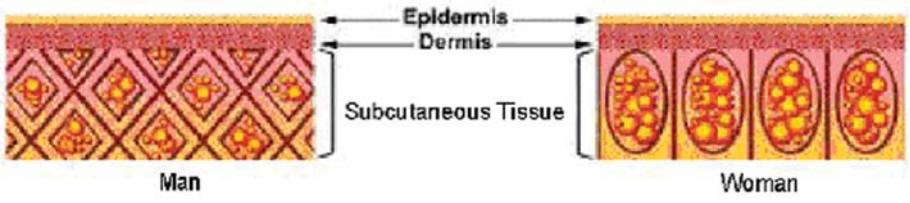
Biochemical interactions in skin are based on a complex balancing system. Any kind of food intake will either be used at once and transferred into energy, or if it is not needed immediately, it will be stored in form of fat in the adipocytes.

The process of using and storing energy is a well defined balance. However, prolonged overeating causes the balance to shift towards fat storage which will result in a change of the fat cell chambers / adipocytes (Figure 1). Cellulite develops when connective tissue between fat cell chambers, and between the chambers and the skin, become thinner and weakened. The elastic fibres that give these connective tissues strength are diminished.

Cellulite or "orange-peel" skin appears predominantly in females[1], due to a different structure of the fat cell chambers. While in the male, elastic fibres give extra strength to the fat cell chambers, female fat cell chambers are less structured (Figure 2). The cells are only formed by the vertical standing cell membranes. Two mechanisms can be used to counteract the appearance of cellulite or "orange-peel" skin.

1. The process of lipogenesis, the fat storage in adipocytes, is based on the hydrolysis of lipoproteins and chylomicrons (circular lipoprotein structures) by the enzyme lipoprotein lipase (LPL) to fatty acids and glycerol (Table 1).
   The fatty acids are stored in adipose tissue as triglycerides.
   The fatty acids are stored in adipocytes as acyl esters of glycerol (triglycerides). The inhibition of LPL should decrease the storage of fat.
2. The decomposition of lipids or lipolysis and the restructuring of collagen fibres can be stimulated by the joint activity of tannins interacting as active vitamin P and methyl xanthines. Several products have become available on the market based on

Figure 2



Treatment	LPL activity (cpm)	sd	n	%	p
Control + LPL	11190	568	3	100	-
Control without LPL	2634	39	3	24	p <0.01
Hibiscus sabdariffa extract + Control +LPL					
10%	2334	139	3	21	p <0.01
5%	3198	72	3	29	p <0.01
2%	6694	828	3	60	p <0.01

Key:
sd = standard deviation
n = number of samples
nd = non-differentiated cells
p = probability

Table 2

Figure 5. Heparin was added to differentiated adipocytes in order to induce the release of LPL. Two controls were used, one without LPL and one using non-differentiated cells.
The release of fatty acid into the medium was measured as LPL activity expressed as % of control and LPL.
The effect of the botanical extract is concentration

tested against a formulation containing 4% of the encapsulated plant extract for 30 days, applied twice a day on the thighs. Before and after the test the circumference of the thighs and the lipid content of the subcutaneous tissue were measured and collated. NMR-analysis was used to determine the lipid content of the subcutaneous tissues.

RESULTS

1. Lipogenesis

The inhibition of LPL activity (from a commercial enzyme) by the specialized *Hibiscus sabdariffa* extract is shown in Table 2 and Figure 4.
The inhibition of LPL activity (extracted from the differentiated 3T3L1 adipocytes) by the specialised *Hibiscus sabdariffa* extract is shown in Table 3 and

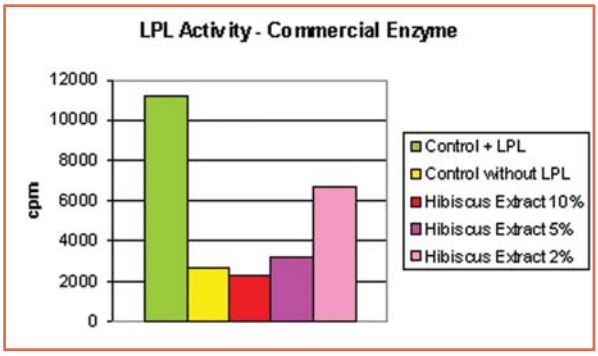


Figure 4

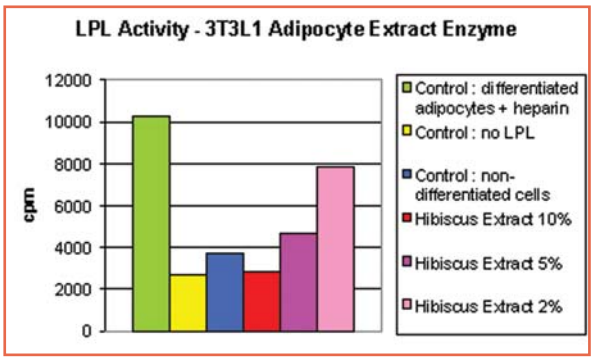


Figure 5

dependent.

2. Decomposition of Lipids

The results have been calculated as a percentage of the differences between both treatments (A: placebo cream / B: test cream). Figure 6 shows the results of the test formulation on the circumference of the thighs after 1 month compared with the placebo (=0).
Up to 3% reduction in the circumference could be observed on 4 of 6 volunteers after 1 month of treatment.
The lipid content of the subcutaneous tissues, measured by NMR, was determined comparing the test formulation containing 4% of the methyl xanthine-rich liposomes with the placebo (=0) (Figure 7).
A reduction of up to 28% of the subcutaneous lipids could be measured. 3 out of 6 volunteers showed a significant reduction of the subcutaneous lipids.

Table 3

Treatment	LPL activity (cpm)	sd	n	%	p
Control + heparin	10261	417	3	100	-
Control medium	2702	50	3	26	p <0.01
Control nd	3717	57		36	
Slimming Factor Karkade + Control					
10%	2873	137	3	28	p <0.01
5%	4688	227	3	46	p <0.01
2%	7843	165	3	76	p <0.01

Key:
sd = standard deviation
n = number of samples
nd = non-differentiated cells
p = probability

CONCLUSIONS

The effect of cellulite can be reduced by the use of cosmetic

Table 1

Extracellular Medium	*Intracellular Medium*
Lipogenesis	
Triglycerides → hydrolysed by **LPL** → (chylomicrons & lipoproteins from diet)	**Fatty acids** + glycerol ↓ **Stored in adipose tissue as Triglycerides**

caffeine, another on methyl xanthine.
The mechanism by which methyl xanthines such as aminophylline and caffeine modify subcutaneous layers has not been systematically illustrated[2]. However, there is research to show that methyl xanthines cause lipolysis, or hydrolysis of adipose tissue into free fatty acid chains[3]. It is also believed that the endocytotic process by which caffeine is absorbed into the cell stimulates the sodium pump to release sodium into the extracellular fluid, causing intracellular dehydration[4,5]. Additionally, the inability to ensure that the methyl xanthines are delivered to the appropriate subcutaneous layer has most likely contributed to the poor results seen in the past with aminophylline and caffeine-based creams. Encapsulating the ingredients in a liposome may ensure the delivery of the methyl xanthines to the appropriate layer, as encapsulated liposomes have been shown to be effective at delivering higher concentrations of ingredients to the subcutaneous layers of the skin[6].

MATERIALS AND METHODS

1. Lipogenesis

The effect of lipogenesis could be demonstrated by using a specialised herbal extract based on *Hibiscus sabdariffa flowers*[7]. The flowers are known to contain fruit acids (15-30%), anthocyanins (approx. 1,5%), ascorbic acid (0.004-0.005%), flavonal glucoside, phytosterols and hibiscic acid (approx. 15%). [Slimming Factor Karkade, Cosmetochem International Ltd., Steinhausen, Switzerland] [8]. The test method used was a cell culture test based on pre-adipocytes. LPL (lipoprotein lipase) is the enzyme that releases the fatty acid from the chylomicrons and the lipoproteins. These fatty acids can be stored in the adipocyte by forming an ester with glycerol. Inhibiting the enzyme reduces the storage of fat. The enzyme used to hydrolyse triglycerides was chosen from two different sources.

- Harvested from laboratory cultured pre-adipocytes (Bioalternatives, Gencay, France)
- Commercial preparation of LPL (SIGMA L2254)

Pre-adipocytes (Strain: 3T3-L1) were cultivated in a medium of 10% DMEM (Life Technologies 21969035) containing 10% of foetal calf serum, changing the serum every 2-3 days. The substrate was a stable emulsion of ^{3}H-Triolein (glycerol trioleate, according to the protocol of Nilsson-Ehle and Schotz) at 250µCi/ml. The specialised extract of *Hibiscus sabdariffa* was used at concentrations of 2%, 5% and 10%. The enzyme, the substrate and the samples were incubated for 90 minutes. The enzyme activity was measured by a scintillation counter. The higher the counts per minute (cpm) the higher the level of free fatty acids and therefore the greater the activity of LPL.

2. Decomposition of Lipids

The decomposition of lipids was shown with an encapsulated plant extract rich in methyl xanthines from the Theaceae botanical family [Slimming Factor T, Cosmetochem International Ltd., Steinhausen, Switzerland] [9,10].
The average size of the liposomes ranged between 150 - 200 nm. The liposomes were paucilamellar (1-2 membranes only), controlled by electron microscopy. The concentration of membrane lipids was high at a level of 6.5% of the product.
The stability of the liposomes were tested by measuring the Zeta-potential (Figure 3). The zeta potential is the overall charge a particle acquires in a specific medium. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. If the particles have low zeta potential values then there is no force to prevent the particles coming together and there is dispersion instability. A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30mV. Particles with zeta potentials more positive than +30mV are normally considered stable. Particles with zeta potentials more negative than -30mV are normally considered stable.
A pilot study of 6 human volunteers has been carried out. 6 healthy females aged between 28 and 43 were selected for the study. A placebo formulation was

Figure 3

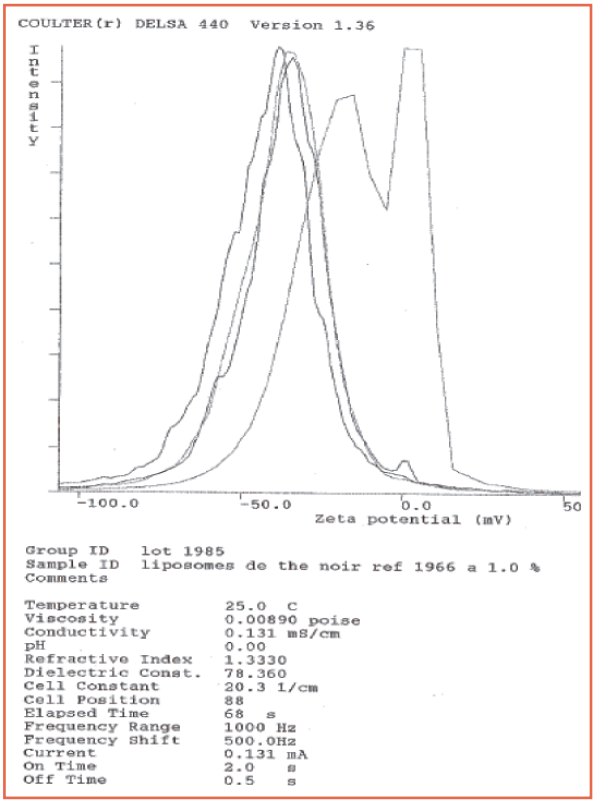


Figure 6

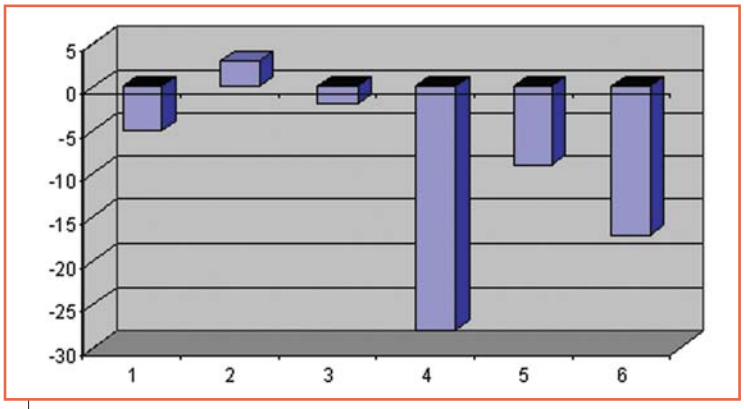
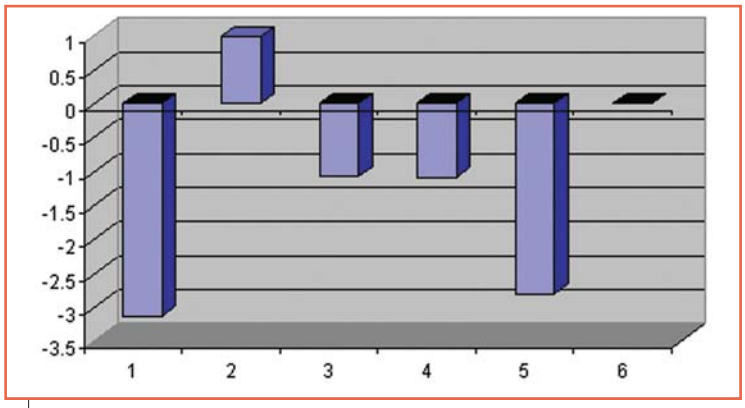


Figure 7



formulations in addition to calorie-restricted diets and specific exercising. Results from both an *in-vitro* test and an *in-vivo* pilot study have shown that two different pathways can be targeted successfully in order to treat cellulite.

The *in-vitro* test focuses on the inhibition of lipoprotein lipase, the enzyme that is responsible for the storage of fat. Pre-adipocytes develop into adipocytes when prolonged overeating forces the body to store the energy that is not immediately needed. The adipocyte morphology in men and women is different. Female fat cell chambers are much less structured which leads to a much more obvious development of the "orange-peel" skin in women compared to men.

Even women with little or no tendency to overweight, develop "orange-peel" skin due to the structure of female fat cell chambers. The inhibition of LPL reduces fat storage at an early state in the extracellular medium. It prevents the fatty acids being esterified for transport via lipoproteins or chylomicrons.

If the fatty acids do not reach the pre-adipocytes, they do not become an adipocyte filled with triglycerides. The appearance of the skin should remain smooth.

The *in-vivo* pilot study is based on the stimulation of lipid decomposition. Lipid decomposition is a process that occurs in the intracellular medium.

Methyl xanthines cause lipolysis, or hydrolysis of adipose tissue into free fatty acid chains. The delivery of methyl xanthines to the subcutaneous layer must be efficient in order to obtain measurable effects.

The encapsulation of methyl xanthines in liposomes provides the necessary transport medium to reach the appropriate layers. Methyl xanthines inhibit the action of the enzyme cyclic 3',5'-nucleotide phosphodiesterase. This enzyme causes the degradation of cyclic adenosine monophosphate (cAMP) into 5'-AMP. Inhibition of the enzyme therefore results in relatively high levels of cAMP, which stimulates a protein kinase that converts the inactive enzyme triacylglycerol lipase into an active lipase.

This causes hydrolysis of triacylglycerols and releases free fatty acids and glycerol into the interstitial fluid and plasma.

The individual studies[7,9] already show measurable effects. The two possible mechanisms to inhibit cellulite formation work in a complimentary way and it could be assumed that a combination of both actives in a cosmetic application would enhance the effect of the single active.

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