Cosmetic Management of Lipid Storage in Adipocytes
A slimming concept for men and women

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Introduction

The unsightliness of “spare tires” and localized fat deposits on thighs (cellulitis), the latter having been for a long time a preoccupation for women, has given rise to the development of numerous cosmetic “slimming” products.

The stimulation of lipolysis (breakdown of triglycerides) by various substances (caffeine, numerous plant extracts, synthetic molecules) constitutes the most common approach in these formulas.

In order to improve on existing products and to propose a mechanism of action that would work independent of gender, we investigated the steps that lead to lipid storage in the adipocytes in the first place and developed a synergistic blend of ingredients that contains Green coffee extract, rich in cafestol and kahweol, and Yerba mate extract, rich in methyl xanthines. They were selected on the basis of in vitro screening tests on human adipocytes.

The two extracts exhibited very interesting properties in vitro. Those properties were subsequently confirmed in the local treatment of fatty overloads (cellulitis) of the thighs in a study on a female panel and on the hips (spare tire) in a study on a male panel. The research and approach to selection of those active substances constitute the subject of the present paper.

Concept and Mechanism

In addition to activating adipocyte fat store depletion (lipolysis), it is possible to deprive the cell of its supply source via the receptors enabling lipids to enter the cells. We are now aware that adipocytes take in the nutrients required for maturation through the intermediary of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). Triglyceride storage is largely related to the fatty acids released by VLDL (Yano et al., 1997).

The adipocyte stores the lipids supplied by the blood following ingestion of a meal using a special type of receptor: clathrin receptors. Following a meal, cholesterol, triglycerides and other lipids are transported in the blood in the form of chylomicrons. After partial hydrolysis, the chylomicrons yield various transport forms, which constitute a system of addressing those forms to the various cell compartments (Valet, 1997). For adipocytes, the address system mainly consists in LDL and VLDL (Yano et al., 1997). Lipoprotein binds to the clathrin receptors and endocytosis of the LDL receptor and VLDL receptor complexes occurs; the triglyceride content and cholesterol transported are then released within the adipocytes.

Cholesterol is a key factor in the regulation of metabolism in the adipocyte. The free cholesterol level in the cell regulates the influx of exogenous cholesterol and endogenous neosynthesis via the mevalonate pathway. The work of the Nobel prize winner J. L. Goldstein (1990) demonstrated that above a certain critical threshold, excessive free intracellular cholesterol exerts a feedback effect blocking over-accumulation: blockade of the synthesis of LDL receptors occurs and the two key enzymes in cholesterol neosynthesis, HMGCoA synthetase and reductase, are downregulated.

Thus, the higher the concentration of free cholesterol in the adipocyte, the more the storage potential for new cholesterol molecules and hence triglycerides (via the influx of LDL and VLDL) is reduced by a reduction in the number of receptors able to bind LDL and VLDL for endocytosis. It will thus be understood that artificially maintaining a high intracellular...
cholesterol level will result in prolonged blockade of exogenous fat influx. Since it is not appropriate to uselessly overload adipocytes with cholesterol, an elegant solution consists in using a cholesterol-mimetic effect with a structural analogue. Screening of a number of cholesterol analogues has allowed us to select kahweol and cafestol as specific plant derived inhibitors of LDL and VLDL receptor synthesis.

Finally, the combination of Yerba mate, a plant derived extract containing titrated amounts of theobromine (1000 ppm) and caffeine (1%) and green coffee extract rich in cafestol (~1000 ppm) and kahweol, constitutes the basic ingredient of a product (trade name UNISLIM™) for lipid management.

In vitro testing:

Stimulation of intra-adipocytic glycerol release
Lipids are stored in the adipocyte as triglycerides. The lipolytic effect was evaluated by determining the quantity of glycerol released by the cell during degradation of triglycerides.

3T3-L1 cells were incubated in the presence of the two xanthines in Yerba mate extract: caffeine and theobromine. At T0, differentiated adipocytes were exposed to rising concentrations of caffeine and theobromine.

Following incubation for 3 hours, the quantity of glycerol was determined by spectrophotometry (\(\lambda = 340\) nm) using an enzymatic method. The percentages reported in figure 1 were determined relative to untreated control cells and the study was validated by comparison with a positive control, isoproterenol at 10^{-6}M.
Inhibition of lipid storage
Neo-adipocytes 3T3-L1 in the adipocyte differentiation phase (after addition of an appropriate triggering cocktail) were incubated with green coffee extract containing cafestol for 24 hours. At the end of the test, the adipocytes were lysed and the extracted mRNA transcribed to cDNA (by reverse transcriptase). The cDNA were then amplified (n copies using Taq polymerase) to obtain a detectable signal.

The specific probes (1 pair of probes per cDNA) for the receptors LDLr and VLDLr were selected from specific databanks so as to generate amplicons of carefully controlled sizes: 249 bp (base pairs) for the LDLr sequence and 204 bp for the VLDLr sequence. This enables simple detection by the RT-PCR quantitation system and visual illustration by electrophoresis.

![Figure 4a: Control adipocytes. Strong fluorescence showing marked LDLr expression](image1)

![Figure 4b: Adipocytes treated with cafestol. Diffuse fluorescence indicating LDLr expression inhibition.](image2)

Figure 4 shows the fluorescence intensity in cultured adipocytes, specifically labelled to detect LDL receptors. Clearly when the cells are treated with cafestol, the expression of LDL receptors decreases, and thus the fluorescence intensity decreases. Quantitative results can be obtained from the RT-PCR experiment. They yield the data given in table 1:

<table>
<thead>
<tr>
<th>mRNA expression</th>
<th>Values standardized on the internal standard</th>
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<tbody>
<tr>
<td></td>
<td>VLDL receptor</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Cafestol acetate (15 ppm)</td>
<td>92</td>
</tr>
</tbody>
</table>

*Table 1: Quantification of mRNA expression in adipocytes incubated with cafestol and green coffee extract for 24 hours.*

These results, obtained under the action of cafestol on the feedback control of LDLr receptor messenger RNA transcription, corroborate the data found in the literature. However, to the best of our knowledge, VLDLr inhibition had not been previously described.
They validate the working hypothesis with respect to the intracellular cholesterol pool as a regulator of adipocyte influx routes via LDLr and VLDLr and open a highly innovative approach to blocking the influx of lipids into adipocytes. It is thus possible to considerably and progressively decrease lipid loading since LDL and VLDL influx account for 60% of intra-adipocyte lipids.

**In vivo studies:**

The *in vivo* efficacy study on UNISLIM™ was conducted on a female panel and a male panel, separately, using the method known as interference-fringe topometry (FOITS). The volunteers, 15 male subjects and 12 female subjects, applied 3% UNISLIM™ cream twice daily for 56 days. Each subject acted as his/her own control and the treated side was compared with the untreated contralateral side.

**Study on the female panel.**
Twelve volunteers of mean age 28.7 ± 8 years took part in the study. Thigh volume was compared at T0 and T56.

![Figure 5: Mean reduction in thigh volume.](image)

After 56 days of application, thigh volume showed very significant changes with a 10% decrease (p<0.01), up to -27%. Over the same period, there was no significant difference for the untreated thighs and even a non-significant very slight increase. Daily use of 3% UNISLIM™ thus demonstrated its ability to reduce fat overload of the thighs in women.

**Study on the male panel**
Fifteen volunteers of mean age 42.3 ± 11 years took part in the study.
Figure 6: Mean reduction in hip volume.

Table 2: Mean changes in “spare tire” amplitude, T0 vs. T56 days, after twice daily application of 3% UNISLIM™ by 15 male volunteers.

<table>
<thead>
<tr>
<th>MALE PANEL</th>
<th>Spare tire amplitude after 56 days of treatment with 3% UNISLIM™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated side</td>
</tr>
<tr>
<td></td>
<td>-17.7</td>
</tr>
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</table>

After 56 days of application, the volume of the spare tire at the hip decreased very significantly (p<0.059) by 17.7 ml. Over the same period, the untreated hips showed no significant difference. UNISLIM™ is the first slimming cosmetic active substance designed for men and women.

CONCLUSION

From the above studies and further considerations it becomes evident that the association of active ingredients is synergistic. In addition, it can be affirmed that using this synergistic combination in cosmetic products leads to further enhancement of lipolysis, cellulite reduction, skin firming and general skin conditioning when used in conjunction with any of the many skin care and body care actives known to the formulator (Sederma, 2004). Non limiting examples include xanthine derivatives (caffeine, theophylline, aminophylline, guarana extracts), organic acids (hydroxycitrate, alpha and beta hydroxy acids), lipolytic peptides, keratolytic substances, products to inhibit adipocyte differentiation (boldine and derivatives, glaucine) and lipogenesis (garcinia extracts).

The use of plant derived, natural cholesterol analogs such as kahweol and cafestol leads, via the inhibition of the synthesis of LDL and VLDL receptors, the entry ports of lipids into the adipocyte, to improved management of lipid storage. The mechanism described and exploited by this concept is not gender dependent, as it does not address the α- and β-adrenergic receptors for which sex related differences have been reported.

References:


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