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An evaluation of mesotherapy solutions for inducing lipolysis and treating cellulite[☆]

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Summary The aim of this study was to evaluate the lipolytic potential of solutions used in the practice of cosmetic mesotherapy to stimulate lipolysis, cause local fat reduction and reduce the appearance of cellulite. The mesotherapy solutions were tested in a human fat cell assay using the fold induction of glycerol generation as a measure of lipolysis. The following mesotherapy solutions were tested: aminophylline; yohimbine; isoproterenol; melilotus; aminophylline with melilotus; aminophylline with isoproterenol; aminophylline with isoproterenol and yohimbine; aminophylline with isoproterenol and lidocaine; and aminophylline with isoproterenol, yohimbine and lidocaine. Isoproterenol ($P < 0.002$), aminophylline ($P < 0.00004$) and yohimbine ($P < 0.001$) stimulated lipolysis compared to the buffer control. The lipolysis stimulated by melilotus ($P < 0.01$) and isoproterenol ($P < 0.002$) was enhanced by aminophylline ($P < 0.001$ and $P < 0.001$, respectively). The lipolytic stimulation by aminophylline and isoproterenol ($P < 0.0009$), and by aminophylline and isoproterenol with yohimbine ($P < 0.0007$) was inhibited by lidocaine, not significant compared to buffer control for aminophylline and isoproterenol, but aminophylline, isoproterenol and yohimbine still stimulated lipolysis more than control, $P < 0.05$).

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Isoproterenol, aminophylline, yohimbine and melilotus stimulate lipolysis alone, and lipolysis is further enhanced by combining lipolytic stimulators in mesotherapy solutions. Lidocaine is antilipolytic and should be removed from mesotherapy solutions designed for local fat reduction. © 2008 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Michel Pistor gave an intravenous injection of procaine to a man suffering from asthma in France during the 1950s, and this experience started the practice of mesotherapy. Although the asthma did not improve, the man's long-standing deafness improved temporarily. Dr Pistor concluded that injection into the subcutaneous tissues would give the best results, and claimed many health benefits from his method of injecting procaine locally. Since the tissue into which he was injecting was of mesodermal origin, he called this technique mesotherapy.¹

Mesotherapy has gained popularity in France and is now included in French medical school curricula. Mesotherapy has recently been gaining in popularity in the United States. The primary application of mesotherapy in the United States has been to induce lipolysis for local fat reduction and to reduce the appearance of cellulite, the lumpy–bumpy, orange-peel texture of the skin on the buttocks and thighs of many women. The use of mesotherapy solutions to induce local lipolysis is primarily based on empirical observations and long-term clinical use, including the incorporation of local anaesthetics like lidocaine or procaine in these mesotherapy solutions. This study evaluates the lipolytic potential of several compounds commonly used separately and together in cosmetic mesotherapy solutions using a human fat cell assay with the generation of glycerol as the measure of lipolysis.

Methods

The lipolysis assay (ZenBio, Research Triangle Park, NC, USA) uses differentiated human adipocytes, which are adherent to the bottom of each well in a 96-well plate, to test the effects of various compounds on lipolysis. The test compounds will either stimulate or inhibit the release of glycerol from the adipocytes relative to the assay buffer, and this difference is stated as a fold induction index. Controls of assay buffer, saline and positive controls of isoproterenol and isobutylmethylxanthine (IBMX) were also included. Table 1 lists the mesotherapy solutions tested and the concentrations at which they were tested, corresponding to concentrations used in the practice of mesotherapy.

Upon arrival of the lipolysis assay kit, 100 μ l of the shipping medium were removed from each well and discarded. Plate A was placed in the incubator for 5–7 days to allow the cells to recover from the stress of shipping. Prior to removing Plate A from the incubator, all test compounds were made by diluting them to their final concentration in Assay Buffer. On the day of the assay, 150 μ l of medium were removed from each well of Plate A, and 200 μ l of Wash Buffer were then added to each well of Plate A. Next 200 μ l of medium and Wash Buffer were removed, and another 200 μ l of

Wash Buffer were added to each well of Plate A. All medium and Wash Buffer were removed from each well of Plate A. The cells were treated with 150 μ l of the test compounds and controls, three wells at a time. The diluted IBMX and isoproterenol were treated as positive controls, and the assay buffer was treated as one of the vehicle controls. The plate was incubated for 5 h at 37 °C, 5% CO₂.

After incubation, 100 μ l of the test compounds were removed and added to a sterile 96-well plate. Glycerol reagent (100 μ l) was then added to create a colorimetric assay dependent on the amount of glycerol released during incubation with the test compounds. A series of glycerol standards were also run with each assay to create a standard curve upon which the results were based. The plates were then read in a spectrophotometer plate reader at 540 nm and the results were plotted based on the standard curve. The results were analysed by *t*-tests and expressed as the mean \pm standard error of the mean (SEM), with the number of wells (observations) noted.

Results

Isoproterenol, aminophylline, yohimbine (Figure 1) and melilotus all significantly stimulated lipolysis compared to the buffer control. The addition of aminophylline to melilotus significantly increased lipolysis compared to melilotus alone (Figure 2). The addition of aminophylline to isoproterenol increased lipolysis significantly compared to isoproterenol alone (Figure 3). The addition of lidocaine to isoproterenol and aminophylline (Figure 4) inhibited lipolysis such that the combination was not statistically different from control. The addition of lidocaine to the combination of isoproterenol, aminophylline and yohimbine (Figure 5) inhibited lipolysis but the combination was still statistically different from control ($P < 0.05$).

Discussion

The most important finding of this study was the inhibition of lipolysis by topical anaesthetics, since almost all mesotherapy solutions have routinely included procaine, lidocaine or some other topical anaesthetic. Evidence exists in the medical literature that local anaesthetics do inhibit lipolysis in human fat cells. Mersmann demonstrated lipolytic inhibition with procaine,² and others have shown that procaine uncouples adenylate cyclase from activating hormone-sensitive lipase, the lipolytic enzyme in fat cells.³ The inhibition of lipolysis by procaine is shared by lidocaine,⁴ and since another topical anaesthetic, prilocaine, also inhibits lipolysis, this seems to be a class effect of local anaesthetics.⁵

Table 1 Stimulation of lipolysis by compounds used in mesotherapy

Components	Concentration	Fold induction (\pm SEM)	P-value	Observations (wells)
IBMX control	1.0×10^{-4} M	2.3 ± 0.24	$P < 0.01$	$n = 3$
Assay buffer	Full strength	1.0	Control	$n = 5$
Aminophylline	1.0×10^{-4} M	2.5 ± 0.57	$P < 0.00004$	$n = 3$
Isoproterenol	1.0×10^{-7} M	2.7 ± 0.06	$P < 0.002$	$n = 3$
Yohimbine	1.0×10^{-7} M	2.0 ± 0.19	$P < 0.001$	$n = 3$
Melilotus	0.02%	2.2 ± 0.33	$P < 0.01$	$n = 3$
Melilotus Aminophylline	0.02% 1.0×10^{-4} M	2.7 ± 0.05	$P < 0.001$ vs control $P < 0.001$ vs melilotus	$n = 3$
Aminophylline Isoproterenol	1.0×10^{-4} M 1.0×10^{-7} M	3.6 ± 0.42	$P < 0.001$ vs control $P < 0.01$ vs isoproterenol	$n = 20$
Aminophylline Isoproterenol Yohimbine	1.0×10^{-4} M 1.0×10^{-7} M 1.0×10^{-7} M	2.5 ± 0.12	$P < 0.0007$ vs control	$n = 3$
Aminophylline Isoproterenol Lidocaine	1.0×10^{-4} M 1.0×10^{-7} M 1.0×10^{-5} M	1.5 ± 0.41	$P = \text{NS}$ vs control	$n = 21$
Aminophylline Isoproterenol Yohimbine Lidocaine	1.0×10^{-4} M 1.0×10^{-7} M 1.0×10^{-7} M 1.0×10^{-5} M	1.4 ± 0.04	$P < 0.05$ vs control	$n = 3$

NS, not significant.

Melilotus is an extract of sweet clover that has been used empirically in mesotherapy to induce lipolysis either alone or in the presence of aminophylline. Our studies confirm that melilotus used alone stimulates lipolysis and that the addition of aminophylline further increases lipolysis.

Isoproterenol, yohimbine and aminophylline are all well recognised lipolytic stimulators.⁶ This study just served to confirm that finding. Isoproterenol has been reported to cause fat loss on one thigh using mesotherapy injections

compared to saline injections on the opposite thigh. Although there are no mesotherapy injection studies demonstrating the clinical efficacy of the other lipolytic stimulators to induce local fat reduction, there are studies using an ointment containing yohimbine, forskolin and aminophylline, an ointment with forskolin alone, an ointment with aminophylline alone, and a cream with aminophylline alone in which the treated thigh lost significantly more girth than the thigh treated with placebo.^{6,7} This suggests that

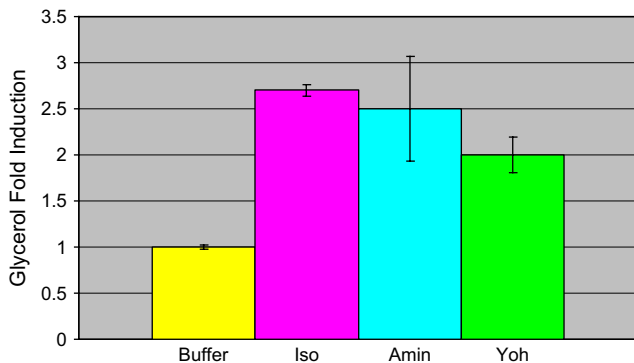


Figure 1 Lipolysis fold induction compared to assay buffer. Iso: isoproterenol, Amin: aminophylline, Yoh: yohimbine.

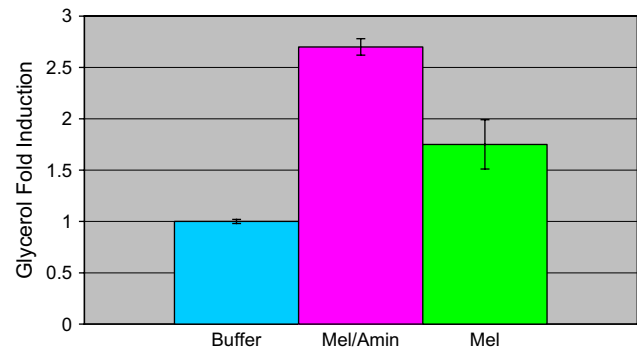


Figure 2 Lipolysis fold induction compared to assay buffer. Mel: melilotus, Amin: aminophylline.

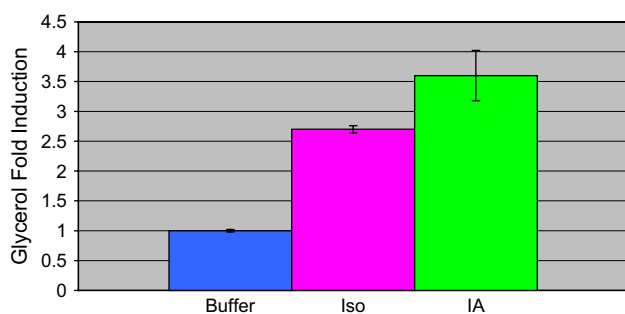


Figure 3 Lipolysis fold induction compared to assay buffer. Iso: isoproterenol, IA: isoproterenol and aminophylline.

delivery of lipolytic agents locally whether by injection or by topical application will cause local fat loss.

Mesotherapists have traditionally combined lipolytic stimulators with the goal of enhancing lipolysis to a greater extent by the use of polypharmacy. Our studies demonstrate that lipolysis induced by melilotus is enhanced by aminophylline as is the lipolysis induced by isoproterenol. Although confirmation will require clinical trials, the enhanced lipolysis in the fat cell assay suggests that combining lipolytic stimulators will enhance local fat reduction in mesotherapy practice.

Cellulite is the descriptive name of the lumpy–bumpy or cottage-cheese appearance that is apparent on the thighs of some women. This appearance of the fat is felt to be the result of the connective tissue architecture of subcutaneous fat, with women having connective tissue oriented at right angles to the skin surface as opposed to men who have a more diagonal orientation of this connective tissue.⁸ Querleux et al.⁹ agree that women with cellulite have connective tissue oriented at right angles to the skin surface, and describe an increase in the deeper adipose layer below Camper's fascia in women with cellulite, but dispute the depiction by Pierard et al.⁸ of the orientation of male connective tissue. Since the appearance is caused by fat cells bulging against these connective tissue strands, emptying out these fat cells by stimulating lipolysis will improve the smoothness of the skin surface. This is a desirable cosmetic effect for many women, and the treatment of cellulite is a major focus of mesotherapy.

Since injecting isoproterenol in the thighs reduces thigh girth and the appearance of cellulite, injection of other lipolytic substances should cause local fat reduction and

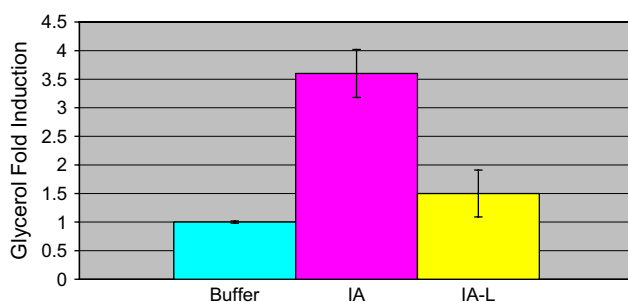


Figure 4 Lipolysis fold induction compared to assay buffer. IA: isoproterenol and aminophylline, IA-L: isoproterenol, aminophylline and lidocaine.

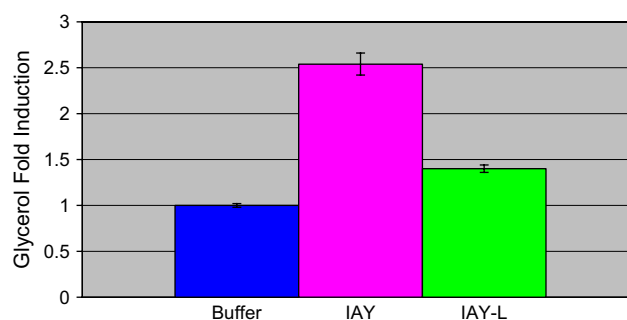


Figure 5 Lipolysis fold induction compared to assay buffer. IAY: isoproterenol, aminophylline and yohimbine, IAY-L: isoproterenol, aminophylline, yohimbine and lidocaine.

improve appearance as well. We have confirmed the lipolytic activity of melilotus, aminophylline, yohimbine and isoproterenol in a human fat cell assay, ingredients which are used for local fat reduction and to reduce the appearance of cellulite in the practice of mesotherapy. We have also demonstrated that combinations of these lipolytic stimulators used in the practice of mesotherapy give greater stimulation of lipolysis than the individual components alone. Most importantly, we have confirmed earlier reports in the literature showing that lidocaine and other topical anaesthetics inhibit lipolysis. We believe that local anaesthetics such as lidocaine and its class derivatives should be removed from mesotherapy solutions designed to cause local fat reduction and reduce the appearance of cellulite.

Acknowledgements

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