

## Study report

### To Study the Lipolytic effect of Actisculpt™ on human adipocyte formation

April 2005

#### Background

Cellulite is typified by the appearance of an orange-peel like skin surface. Cellulite is a particularly female problem, although it can affect men, and it is estimated that 85% of post-adolescent women have cellulite, mainly caused by a combination of hormones, diet and the natural pattern of fat deposition. The increasingly sedentary lifestyles, along with a poor diet, are leading to a general increase in body weight with the resulting increase in appearance of cellulite. The result is that more women are now seeking more effective cosmetic ways to eliminate cellulite.

Caffeine is traditionally used in cosmetics to reduce the appearance of cellulite. We have now developed a unique ingredient called Actisculpt™, which offers an alternative to caffeine or can also be used in combination with it.

Actisculpt™ contains two plant extracts, with known actives, which have been shown to lower fat levels. The plant extracts are Coleus Forskohlii Root extract and Commiphora Mukul Resin extract, and the known actives are standardised within the Actisculpt™ to ensure batch-to-batch consistency.

Coleus Forskohlii Root extract contains the active forskolin, one of many diterpenes found in the coleus species (a small perennial shrub, a member of the mint family). Forskolin works by stimulating adenylate cyclase and intracellular cyclic AMP (cAMP), to elicit cAMP dependent responses in the cell (see over).

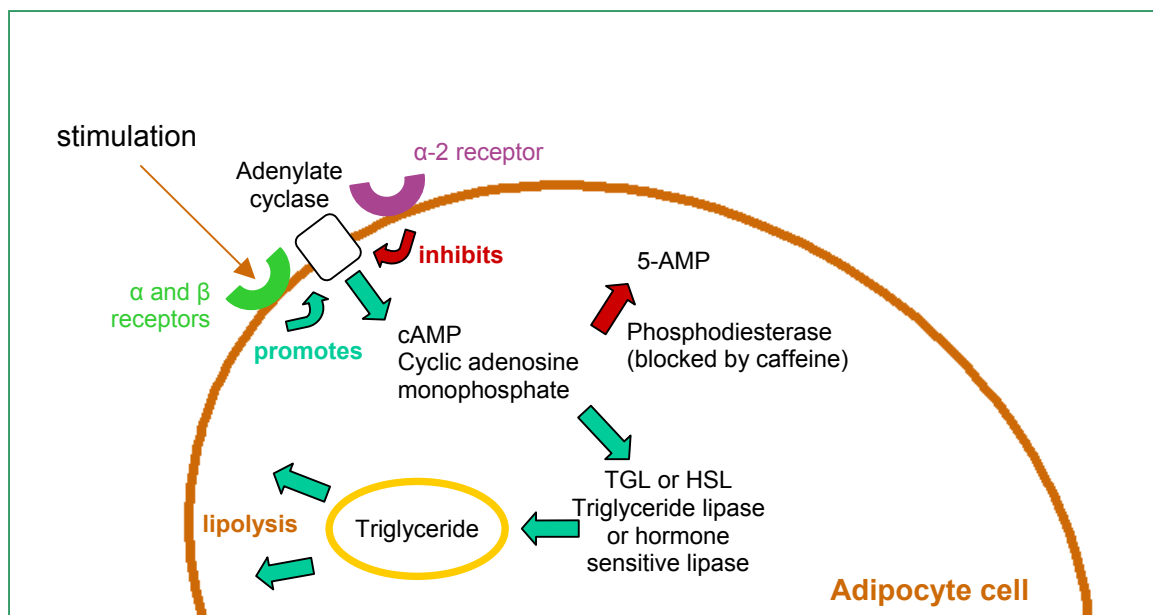
Commiphora Mukul Resin extract is the gum resin from the mukul myrrh tree, a small, thorny tree found in Arabia and India. Traditionally, this plant was used in Ayurveda [Indian] traditional herbal medicine, where it was used for the treatment of obesity and lipid disorders. More recently, it has been shown to significantly lower serum triglycerides and cholesterol as well as LDL and VLDL cholesterols. The active constituents of Commiphora Mukul are the guggulsterones.

## Introduction

The following study has been designed to examine the lipolytic effect shown by the blend of herbal actives on human differentiated adipocytes.

Human differentiated adipocytes were obtained from processed pre-adipocyte cells. A negative control, which consists of pre-adipocyte cells, was also included in the study.

Figure 1 represents a schematic drawing of the major pathway of cAMP stimulation. It shows that an effective anti-cellulite active should increase cAMP, glycerol release and  $\beta$ -oxidation without affecting the cell viability.



**Figure 1:** Representative schematic drawing of a human adipocyte showing the lipolytic pathway in response to stimulation from a cAMP inducer

The study was divided into two parts:

- Cytotoxicity
- cAMP activation study

## **Cytotoxicity**

The cytotoxicity assay was performed on fibroblast (HDF-N2) cells in order to quantify the non-cytotoxic range concentrations of the tested blend.

Data from the cytotoxicity assay using the blend active mix indicated that the optimal final working concentration to be used in cell culture test was 0.001% by wt. From this assay, four concentrations of the tested bend were used in the cAMP activation study (0.002%, 0.001%, 0.0001% and 0.00001%)

## **cAMP Activation Study**

The cAMP level in the adipocyte is an important biochemical indicator for the lipolytic activity of the cell. An increasing cAMP level is an important biochemical indicator for the increase of lipolytic activity (or lipid degradation).

All experimental conditions were run following a standardised procedure.

The assay included two types of controls:

- (i) Non-treated Cells:-  
A negative control consisting of non-treated, differentiated cells (labelled C-\_-ISO 1\_PLA) and non treated, non-differentiated cells (labelled C+\_+ISO 1\_PLA).
- (ii) Treated Cells:-  
A positive control consisting of differentiated cells treated with isoproterenol, a cAMP stimulator (labelled C+\_+ISO1) and a negative control consisting of pre-adipocytes incubated in the presence of isoproterenol (labelled C-\_-ISO1).  
Note that isoproterenol is known to induce lipolysis by increasing the level of c AMP in the cell.

The four test concentrations were labelled C1- C4.

	% Actisculpt	pmol cAMP / mg cell protein	error bar
C1	0.002	335.3	7.1
C2	0.001	256.1	15.8
C3	0.000	36.6	3.6
C4	0.000	14.5	3.1
Controls:			
C-_-ISO1		14.3	0.0
C+_+ISO1		82.6	11.9
C-_-ISO 1_PLA		30.4	16.6
C+_+ISO 1_PLA		34.7	21.3

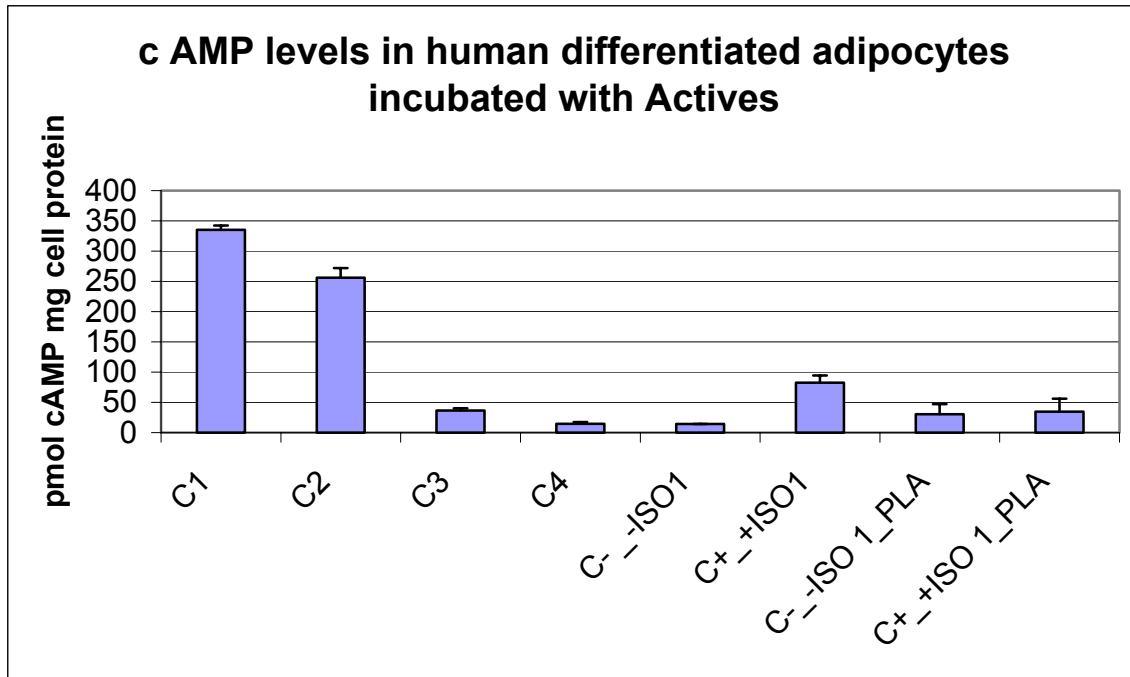


Fig 2: Effects on cAMP synthesis induction

### **Conclusions - cAMP Activation Study**

No significant cAMP synthesis was induced in the undifferentiated cells [negative controls] (labelled C-\_-ISO1 and C+\_+\_ISO1\_PLA).

Significant cAMP synthesis induction by adipocytes could be observed only when isoproterenol [positive control] or the two higher concentrations of the active blend (0.002% and 0.001%) were added to the cell culture.

The two highest assay concentrations of active blend in the cell culture induced a high lipolytic activity as shown by their significant increase of cAMP levels (8-fold and 11-fold respectively) compared to the negative control (C-\_-ISO1\_PLA).

The two highest concentrations of the blend showed a higher apparent efficacy for cAMP synthesis induction (3-fold and 4-fold respectively) when compared to the isoproterenol positive control (C+\_+\_ISO1).

The level of cAMP induced by the two lower concentrations of the active blend was not significant versus the controls.