

Use of the microdialysis technique to assess lipolytic responsiveness of femoral adipose tissue after 12 sessions of mechanical massage technique

C. Monteux*, M. Lafontan**

*LPG Systems, 2753 Route des Dolines BP 243 06905 Sophia Antipolis (France)

** Unité Inserm-UPS 586, IFR-31-Institut Louis Bugnard, Hôpital Rangueil,
BP84225, 31432 Toulouse cedex 4 (France)

Corresponding author:

MONTEUX Clelia

LPG Systems,

2753 Route des Dolines BP 243

06905 Sophia Antipolis (France)

Telephone: 00 33 4 92 38 39 32

Fax: 00 33 4 92 96 09 65.

Email: clelia.monteux@lpgsystems.com

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Abstract

Background: Adipocytes in femoral areas are known to be metabolically “silent”. Changes related to fat cell hypertrophy may be involved in the formation of cellulite. A mechanical massage technique, with circulatory and dermatrophic properties, has been shown to have an impact on clinical evaluations (i.e. changes in morphometric measurements) in cellulite areas. Whether this technique affected lipolytic responsiveness in subcutaneous adipose tissue of cellulite areas was not known.

Objective: Using a microdialysis technique in subcutaneous adipose tissue, a study was carried out to test the *in situ* incidence of a mechanical massage technique in terms of adipose tissue responsiveness to a lipolytic challenge.

Materials and methods: Nine healthy women volunteers with cellulite (grade ≥ 2) were included and treated with 12 sessions of mechanical massage technique (Endermologie®). Microdialysis has been carried out in the femoral adipose tissue in order to assess lipolytic responsiveness via glycerol determination following perfusion of a lipolytic agent (0.1, 1 and 10 μ M isoproterenol). Clinical evaluations (measurements of waist, thighs and skin fold) were carried out in parallel. All evaluations were performed before and after treatment.

Results: The studied intervention lowered resting dialysate glycerol levels in femoral adipose tissue. The lipid-mobilizing effect of isoproterenol was enhanced after 1 month of treatment. In addition, a clear decrease of morphometric measurements (mean decrease on thighs perimeter: 3.1 to 3.3 cm, $p < 0.01$) was observed.

Conclusion: These results suggest an increase in the lipolytic responsiveness of femoral adipose tissue in women with cellulite having undergone 12 sessions of mechanical massage.

Key words: microdialysis, cellulite, femoral adipose tissue

Introduction

Lipolysis in human adipocyte is mediated by the sympathetic nervous system activation. Norepinephrine and epinephrine stimulate both the α 2- and β -adrenergic receptors (AR) on the fat cell surface. Differences in the responsiveness of adipose tissue to the adrenergic stimulation are regional and gender related and related to the functional balance between α 2- and β -adrenergic responses (1-5). In women, lipid accretion is favoured in the gluteo-femoral depots (6) which are characterized by the abundance of α 2-AR and a relatively sluggish fat turnover (3). Increased expression of α 2-AR - and concomitant decrease of β -AR responsiveness - with fat cell hypertrophy could be a physiological adaptation that leads to the reduction of the lipolytic responsiveness of the hypertrophied adipocyte (4). In addition, blood flow was weaker in femoral than in abdominal fat deposits (7, 8). It remains to establish if the defective lipolytic response and reduced local blood flow (which is supposed to lead to some fluid stagnation) in those specific fat areas are involved in the aetiology of cellulite.

A mechanical massage technique with circulatory and dermatrophic properties (9-12) has been shown to have an impact on clinical parameters (i.e. morphometric measurements) in cellulite areas. Whether this technique affected lipolytic responsiveness in subcutaneous adipose tissue (SCAT) of cellulite areas was not known.

Mechanism of lipid mobilization in SCAT can be monitored by microdialysis technique (13). It consists of introducing small microdialysis probes in adipose tissue in order to continuously monitor glycerol (marker of lipolysis) in the extracellular space and at the same time deliver drugs locally to this space, thereby eliminating the systemic effects of the drug (14). This *in vivo* and *in situ* approach allows for the study of adipose cells in their actual milieu and has been largely used. The role of β - and α 2-AR in the control of lipolysis in human adipose tissue (15, 16) and the lipolytic responsiveness of adipose tissue in lean and obese subjects (17) have been studied using this technique through measurement of glycerol output combined with estimation of local blood flow changes as it plays an important role for regulating lipid mobilization (15).

The aim of the present study was to explore the lipolysis regulation of subcutaneous femoral adipocytes in female volunteers subjected to 12 sessions of a mechanical massage technique. Isoproterenol-mediated lipolysis and adipose tissue blood flow were assessed, using *in situ* microdialysis, before and after treatment. Clinical parameters (perimetry and skin fold measurements) were also investigated to appreciate morphological changes.

Material and methods

Subjects:

The experimental procedure was approved by the Local Ethics Committee of Toulouse Hospital (France) and was carried out on nine healthy female volunteers who signed an informed consent form and had cellulite of the thighs of grade ≥ 2 according to Nurnberger classification (18). In order to exclude any interference due to hormonal or environmental effects, the subjects were required to be receiving oral contraception for more than 3 months and to maintain stable dietary habit, weight and physical activity, for the total duration of the study. The characteristics of the subjects are depicted in Table 1.

Experimental design

Before and after treatment, all the evaluations were carried out in the laboratory. Lipolytic response in femoral adipose tissue and local blood flow responsiveness were assessed *in situ*

by dosage of glycerol and urea output respectively, using the microdialysis method. Objective physical measurements included perimetry and skin fold thickness.

Treatment with the mechanical massage technique

The mechanical massage technique (Endermologie®) is a non-invasive technique consisting of a deep tissue mobilization provided by a medical device composed of a treatment chamber with an aspiration system and two independent motorized rollers which roll and unroll a skin fold. Each subject received a total of 12 sessions (thrice a week) of mechanical massage technique according to a specific practical protocol.

Microdialysis experiments

Subjects were investigated at 8:00 a.m. after an overnight fast before and after 12 sessions with the mechanical massage technique. After light epidermal anaesthesia (Emla® patch 5%, AstraZeneca, 20 min.), one microdialysis probe (20 x 0.5 mm, 20-kDa molecular-weight cutoff; CMA Microdialysis, Sweden) was inserted percutaneously into the femoral SCAT at two-thirds of the distance between the patella and the superior anterior iliac spine. The probe was connected to a microinjection pump and continuously perfused with a sterile Ringer solution (154 mM sodium, 6 mM potassium, 2.5 mM calcium, 160 mM chloride). The perfusion was set at a flow rate of 2 µl/min and fractions were collected following a 30-min equilibration period. After fractions were collected for definition of baseline values, the probe was infused with increasing concentrations of the β-AR agonist isoproterenol (0.1; 1 and 10 µM, in Ringer solution). Three 10-min fractions were collected for each concentration and frozen (-80°C) until dosage. Stimulation of lipolysis promotes glycerol appearance in the extracellular fluid which is taken up by the microdialyse probe. Glycerol which is not re-utilized by fat cells (i.e. due to glycerol kinase defect) is the best metabolic index for lipolysis determination (13).

Biochemical determinations

The concentrations of glycerol and urea in the microdialysis samples were analysed using enzymatic methods as previously described (19). Urea was used as an endogenous reference compound to determine adipose tissue blood flow (20).

Perimetry and cutaneous fold

Perimeter measurements were performed with a millimeter tape measured in one point of each thigh (middle thigh) and at the waist and hips. Caliper gauge measurements of skin fold thickness were performed at one lateral point of the right and left inferior limbs. The height of each measurement point was identified throughout the study using a height gauge.

Statistical analysis

All the values are given as means+ SE. Wilcoxon's or Student tests for paired values were used for comparison. P<0.05 was considered statistically significant. Concentration-dependant responses to isoproterenol infusions were compared using an ANOVA for repeated measures. All statistical comparisons were performed by using a statistical software package (Stata SE 8.2, Statacorp, College Station, Texas, USA).

Results

All patients (nine) completed the 12 sessions. The sample size for analyses was 9, except for the microdialysis measurements where it was 8, due to a technical problem with one patient.

Changes of lipid-mobilizing responses to isoproterenol induced by the mechanical massage technique in femoral adipose tissue

The effect of isoproterenol on glycerol output is depicted in Fig.1.

At basal level (without isoproterenol), glycerol concentration is lowered after treatment but the difference is not statistically significant ($p=0.0882$).

The addition of isoproterenol into the perfusate increased the dialysate glycerol concentration over the baseline significantly at $1\mu\text{M}$ before treatment ($p = 0.0108$) and at 0.1 and $1\mu\text{M}$ after treatment ($p = 0.0038$ and 0.0153 respectively).

The highest isoproterenol concentration ($10\mu\text{M}$) did not significantly modify the glycerol output in any condition.

Results expressed in percentage over the baseline value are shown in Fig 2. After 12 sessions with the mechanical massage technique, responsiveness to isoproterenol infusion is improved for the usually used concentrations in microdialysis experiments on adipose tissue: 50% versus 27% at $0.1\mu\text{M}$, 83% versus 63% at $1\mu\text{M}$ (ANOVA $p = 0.0441$) and 37.5% versus 0% at $10\mu\text{M}$ (ns), respectively for responses obtained after and before treatment.

Effects of the mechanical massage technique on femoral adipose tissue blood flow to isoproterenol.

The effect of isoproterenol on urea concentration is depicted in Fig.3.

At basal level (without isoproterenol), urea concentration is similar before and after treatment (0.82 ± 0.47 versus 0.78 ± 0.39 mmol/l, ns)

The addition of isoproterenol into the perfusate did not significantly modify the urea concentration when compared to baseline except at the highest concentration used ($10\mu\text{M}$) before treatment and at $1\mu\text{M}$ after treatment where it is lowered ($p = 0.004$ and $p = 0.0102$ respectively).

When results are expressed in percentage below the baseline, isoproterenol infusion caused a dose-dependant decrease in urea concentrations from 8.5% at $0.1\mu\text{M}$ ($p = 0.2084$) to 18.3% at $1\mu\text{M}$ ($p = 0.1451$) and 46.3% at $10\mu\text{M}$ ($p = 0.004$) before treatment. The decrease in the urea concentrations promoted by 0.1 and $1\mu\text{M}$ isoproterenol is greater after treatment (23% and 25%, respectively) but it is not statistically significant ($p = 0.111$ and $p = 0.257$ respectively).

Effects of the mechanical massage technique on clinical evaluations

Statistical comparison of morphologic parameters are summarized in Table 2. On the whole, the measurements showed a significant reduction after treatment. This reduction is observed on cutaneous fold (-12.89% on the Right side and -13.41% on the Left side), waist and thigh measurements (1.7 to 3.3 cm; $p<0.01$).

Discussion

The main finding of this study is that treatment of femoral fat deposits with 12 sessions of mechanical massage technique promoted an increase β -AR-mediated responsiveness in a tissue known to be poorly responsive to catecholamines and metabolically "silent" in terms of non-esterified fatty acid release (8). Before treatment, the β -AR agonist at $1\mu\text{M}$ promoted a weak but significant stimulation of glycerol output. It is the response expected in a tissue known to be poorly responsive to lipolytic agents (8). It must be noted that the highest pharmacologic concentration of isoproterenol (e.g. without physiological relevance) is without effect. After treatment, isoproterenol responsiveness was improved as shown by a

shift to the left of the concentration-response curve, 0.1 μM isoproterenol becoming efficient. Moreover, percent increase over basal values was enhanced after treatment. Although they had no quantitative relevance, urea results suggest that blood flow changes also occur after treatment with the mechanical massage. However, interpretations are not easy in the absence of further investigations with another blood flow tracer. The limited number of patients involved in the present study allows a clear demonstration of the metabolic impact of this mechanical massage. Nevertheless, it was not possible to correlate isoproterenol-induced lipolytic responses of adipose tissue and the morphologic change; such a study, which requires a large number of patients, was considered to be out of the scope of the present investigation.

The biphasic concentration response-curve depicted in the figures, when infusing isoproterenol, is difficult to interpret in the absence of more detailed studies of the composition of dialysate. It must be noted that this kind of biphasic curve is obtained when treating isolated fat cells with isoproterenol or norepinephrine *in vitro* (21). Sustained stimulation of lipolysis by pharmacological doses of lipolytic agents induces ATP depletion and reduces lipolytic responses. Moreover, since the effects of isoproterenol are performed with sequential and cumulative procedure, it is not excluded that a partial desensitization of β -AR (1), leading to a reduction of the response, could occur under our working conditions. When performing studies *in vivo*, it is also possible that at the highest concentration, isoproterenol treatment alters the normal function of adipocytes and endothelial cells. It is possible that infusion of the higher doses strongly alters adenosine and prostaglandin release, two agents known to exert anti-lipolytic effects and possess vascular actions. All these factors could alter glycerol output from the microdialysis probe.

Cellulite is a condition that remains an issue of cosmetic concern to a great number of women. A large part of the difficulties in treating cellulite arises from our incomplete understanding of this phenomenon (22-24). Questions concerning aetiology and even definition of cellulite remain largely open and debated. Due to the lack of convincing experimental studies, there is no consensus.

A number of products (herbal extracts, retinol, caffeine, ruscogenin,...) with lipolytic, venotonic or trophic properties, are used in numerous slimming treatments (topics, dietary complements, mesotherapy) (25-29). Modern technological advances using laser or electromagnetic energies claim to improve cellulite (30, 31), but data are mainly subjective. Finally, efficacy and safety of anti-cellulite treatments described in literature are more or less convincing (22).

The present investigation is the first attempt to characterize the putative functional impact of a treatment with a mechanical massage technique on the responsiveness of the adipose tissue located in cellulite areas. For that purpose, using the microdialysis technique, the lipolytic responses induced by a β -AR agonist in femoral adipose tissue was investigated before and after treatment in women with cellulite. Similar findings (i.e. improvement of the response promoted by isoproterenol in SCAT) were observed in previous studies carried out in abdominal area of obese subjects submitted to physical exercise (32-35) or hypocaloric diets (36-37). Benefits of exercise training have been explored after long training periods. For example, a 4-month endurance training program, improves ANP- as well as β -AR-mediated lipid mobilization and adipose tissue blood flow in the abdominal SCAT of overweight subjects. The recovery of a higher lipolytic efficiency in adipose tissue is an important benefit of a training program in overweight subjects (34). In front of the results obtained after longer periods of endurance training, it is not excluded that longer sessions or longer periods of treatment with the mechanical massage could be more beneficial for a better recovery of lipolytic responsiveness in femoral fat deposits.

In the absence of further investigations and notably follow-up experiments, it is difficult to speculate on the duration of the improvement observed in the lipolytic responsiveness of femoral adipose tissue. Regarding morphological changes in cellulite areas, a remanent effect has been evaluated at 6 months with perimetry, skin imprints and 20-MHz sonography (11). Nevertheless, based on results of clinical studies in patients performing exercise (34, 35), the improvement observed in the present study is expected to remain efficient in the long term if and only if the patients maintain some physical exercise and nutritional control.

Conclusion

This study shows that a treatment (12 sessions) with this mechanical massage technique improves the lipid-mobilizing effects of *in situ* administered isoproterenol in femoral adipose tissue of women with cellulite. In addition to these functional improvements in femoral adipose tissue biology, the treatment reduced body circumferences and skin fold measurements. The mechanism of action of such treatment is still unclear. It may initiate local changes (i.e. adipose tissue remodelling events) which have an incidence on the lipolytic responsiveness of femoral adipose tissue.

Complementary studies are necessary to confirm the effect in other fat deposits and to enter more deeply into further mechanistic consideration on the incidence of this mechanical massage technique on lipid mobilization in fat deposits.

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Table 1: Characteristics of the subjects:

Characteristics of the subjects	Mean \pm SE	Range
Age, yr	41.2 \pm 3.6	36-45
Height, cm	164.89 \pm 8.34	155-180
Weight, kg	74.37 \pm 13.44	60-97
Body mass index, kg/m ²	27.16 \pm 0.95	22.58-29.94

Table 2: Circumference and cutaneous fold measurements before and after treatment (12 sessions)

	Before treatment	After treatment	<i>p</i>
Waist measurement (cm)	97.8 ± 3.6	96.1 ± 3.6	<0.01
Hip measurement (cm)	108.7 ± 4.0	108.6 ± 4.0	NS
Right thigh measurement (cm)	64.7 ± 3.8	61.4 ± 3.0	<0.01
Left thigh measurement (cm)	64.7 ± 3.8	61.6 ± 3.0	<0.01
Right cutaneous fold (mm)	28.4 ± 1.7	24.8 ± 1.4	<0.001
Left cutaneous fold (mm)	28.4 ± 1.7	24.6 ± 1.4	<0.001

Data are expressed as means + SEM. *P* values were calculated using the Student test

Figure 1: Effect of the nonselective β -adrenoceptor agonist isoproterenol on the dialysate glycerol concentration in femoral SCAT from women subjects (n=8) before and after treatment (12 sessions).

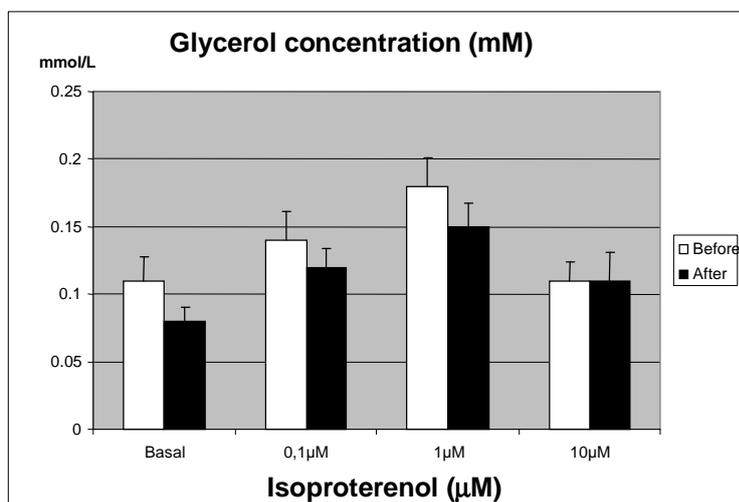


Figure 2: Effect of the nonselective β -adrenoceptor agonist isoproterenol on the glycerol evolution, expressed in % over the baseline value, in femoral SCAT from women subjects (n=8) before and after treatment (12 sessions).

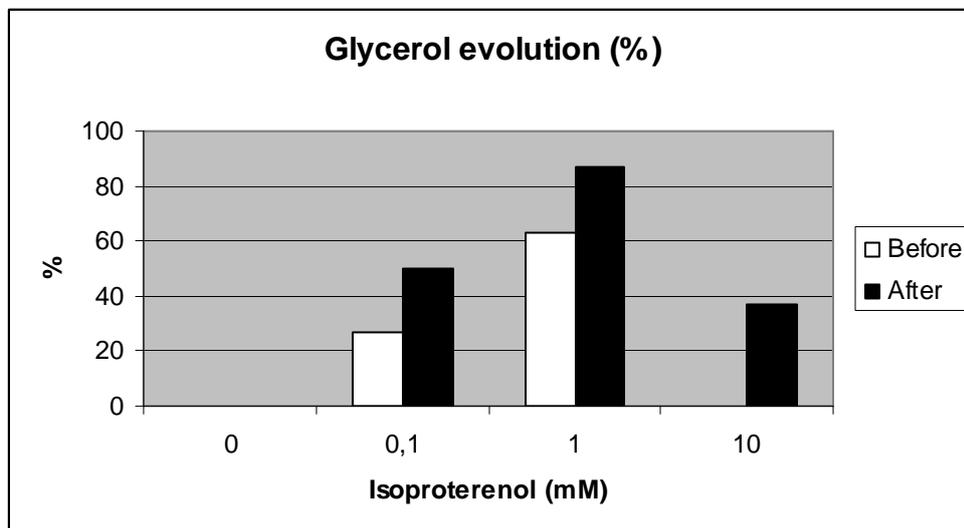


Figure 3: Effect of the nonselective β -adrenoceptor agonist isoproterenol on urea concentration (endogenous reference compound) in femoral SCAT from women subjects (n=8) before and after treatment (12 sessions).

