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Glycyrrhetinic acid, the active principle of licorice, can reduce the thickness of subcutaneous thigh fat through topical application

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Abstract

Cortisol is involved in the distribution and deposition of fat, and its action is regulated by the activity of 11β-hydroxysteroid dehydrogenase. Glycyrrhetinic acid, the active principle of licorice root, blocks 11β-hydroxysteroid dehydrogenase type 1, thus reducing the availability of cortisol at the level of adipocytes.

We evaluated the effect of topical application of a cream containing glycyrrhetinic acid in the thickness of fat at the level of the thigh.

Eighteen healthy women (age range 20–33 years) with normal BMI were randomly allocated to treatment, at the level of the dominant thigh, with a cream containing 2.5% glycyrrhetinic acid ($n = 9$) or with a placebo cream containing the excipients alone ($n = 9$). Before and after 1 month of treatment both the circumference and the thickness of the superficial fat layer of the thighs (by ultrasound analysis) were measured.

The circumference and the thickness of the superficial fat layer were significantly reduced in comparison to the controlateral untreated thigh and to control subjects treated with the placebo cream.

No changes were observed in blood pressure, plasma renin activity, plasma aldosterone or cortisol.

The effect of glycyrrhetinic acid on the thickness of subcutaneous fat was likely related to a block of 11β-hydroxysteroid dehydrogenase type 1 at the level of fat cells; therefore, glycyrrhetinic acid could be effectively used in the reduction of unwanted local fat accumulation.

Keywords: Glycyrrhetinic acid; Topical application; 11β-Hydroxysteroid dehydrogenase type 1; Ultrasound analysis; Subcutaneous fat thickness; Aldosterone; Plasma renin activity; Thigh circumference

1. Introduction

The history of licorice as a medicinal plant is very old and an effect of licorice on the modulation of food and water intake has been reported by Theophrastus in the 4th century BC. This Greek doctor cited licorice root as producing freedom from hunger and thirst. Plinius also reported the same concept in the 1st century BC [1]. In the recent years, several authors have demonstrated an involvement of licorice in the metabolism of cortisol [2,3].

Glucocorticoids play a crucial role in the regulation of deposition and distribution of fat. The activity of cortisol is modulated by 11β-hydroxysteroid dehydrogenase (11HSD), which catalyzes the interconversion of cortisol and cortisone [2]. Two isoforms of 11HSD have been characterized: the type 1 (11HSD1) is predominantly a reductase, reactivating inactive cortisone to cortisol; while the type 2 isoform (11HSD2) inactivates cortisol to cortisone. The latter isoform is involved in the physiological action of aldosterone at the level of classical target tissues [2–5]. When the enzyme is inactive, as happens in apparent mineralocorticoid excess syndrome, a severe low-renin low-aldosterone hypokalemic hypertension develops [2,5]. Prolonged administration of

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glycyrrhetinic acid (GA), the active principle of licorice root, can produce a hypokalemic hypertension by blocking 11HSD2 at the level of epithelial target cells and by binding directly to mineralocorticoid receptors when its plasma concentration is high [6,7].

We have recently demonstrated that licorice, taken orally, can also reduce body fat mass as measured by bioelectrical impedance analysis, possibly by blocking 11HSD1. This effect was not accompanied by a reduction in body mass index (BMI) due to contemporary sodium and water retention [8].

11HSD1 is expressed in both adipocytes and stromal cells (preadipocytes) and its activity, in vitro and in vivo is much higher in adipose stromal cells of the omentum than in subcutaneous adipose tissue11HSD1 [9]. The involvement of cortisol in the distribution and deposition of fat has been widely recognized and the term Cushing's syndrome of adipose tissue has been employed to explain visceral obesity [9,10].

Abnormal metabolism of cortisol is involved in the pathogenesis of obesity: the enzyme 11HSD1 is expressed in target tissues for cortisol and in particular in fat, having predominant reductase activity, thus allowing a greater local availability of cortisol [9,10]. An excess of fat is possibly related to genetic over-expression of 11HSD1, which is involved in the differentiation of preadipocytes into adipocytes both at the visceral and subcutaneous level [10].

In humans it has been shown that abdominal subcutaneous adipose tissue anatomically can be divided into two physiologically different layers and in a recent study it has been shown that the deep layer behaves metabolically like the intra-abdominal fat depot [11,12].

The aim of this study was to evaluate the efficacy of topical application of GA in reducing local fat accumulation at the level of the two fat layers of thigh.

2. Experimental

Eighteen healthy, non-obese female volunteers, age range 20–32 years, were recruited from medical students and from the staff of our Department. The procedures followed were in accordance with the ethical standard of the local Ethic Committee. The subjects were regularly menstruating, were fully informed about the experimental design and gave consent to be studied. None of the subjects were taking contraceptives or other drugs, neither before nor during the experiment and they were following a normocaloric diet without salt restriction.

The following parameters were evaluated before the study: BMI, blood pressure, plasma renin activity (PRA), aldosterone, cortisol, serum potassium and the circumference of both thighs at two premarked levels (at the base and at mid-thigh). The thickness of the fat on a premarked point of the thigh was also evaluated by ultrasound analysis (ESAOTE AU3, probe 7.5 or 10 MHz) for the superficial and deep fat layers (mean of three measurements). Both the subjects and the operator, who performed all the measurements and ultrasound analysis, were blind to the type of treatment.

Blood pressure was measured in sitting position with a Riva-Rocci sphygmomanometer.

Commercial radioimmunoassay kits (Medical System, Italy) measured PRA and plasma aldosterone and cortisol.

The subjects were randomly assigned to two treatment arms: nine were treated on their dominant thigh with the cream containing 2.5% GA (daily application 80 mg) and the other nine with a placebo cream which contained all excipients except GA. The excipients of the cream were: water, glycerin, octyl stearate, cetearyl alcohol, carbomer, disodium EDTA, propylene glycol, glyceryl stearate, methylparaben, ceteareth-12, propylparaben, PEG-40 castor oil, sodium dehydroacetate, 2-bromo-2-nitropropane-1,3-diol, ethylparaben, phenoxyethanol, sodium hydroxide, sodium cetearyl sulfate (NewFields, Padua, Italy).

The two groups were homogeneous for BMI and age. All the measurements were done in the luteal phase before therapy and after application of the cream, in the luteal phase of the subsequent cycle. The subjects were instructed not to change diet and physical activity during the period of evaluation.

Data are represented as mean ± S.E. Statistical analysis was done by Wilcoxon Matched-Pairs Signed-Rank Test (two-tailed) when comparing data from the same subject. Statistical analysis with Mann–Whitney test for unpaired data (two-tailed) was used when comparing the effect of the cream on the GA-treated limb versus placebo-treated limb. A p value of less than 0.05 was considered as statistically significant.

3. Results

Body weight (in GA-treated subjects, from 60.5 ± 0.6 to 60.2 ± 0.6 kg, in placebo-treated subjects from 59.2 ± 0.4 to 59.0 ± 0.4 kg) and BMI (in GA-treated subjects from 21.2 ± 0.2 to 21 ± 0.2 kg, in placebo-treated subjects from 21 ± 0.3 to 20.9 ± 0.3 kg) did not change after treatment ($p > 0.05$). Blood pressure (systolic/diastolic) was similarly not modified after the treatment with GA cream (from 120 ± 1.7.2/84 ± 1.6 to 120 ± 2.1/82 ± 1.4 mmHg).

Fig. 1 shows the different layers of subcutaneous fat at the level of the thigh in one of our subjects. The superficial layer shows in its context a fibrous septum, which is not always clearly distinguishable. The separation between the superficial layer and the deep layer is instead always clear.

The individual values of three determinations using ultrasound analysis within ±2% of the mean value.

We found a significant reduction in the superficial fat layer thickness in the GA cream-treated thigh, both in relation to the contralateral untreated leg and to the placebo-treated ones (Table 1, $p < 0.005$).

Fig. 2 presents the absolute difference between the single superficial fat thicknesses from each subject (value after treatment minus value prior to treatment, both with GA cream

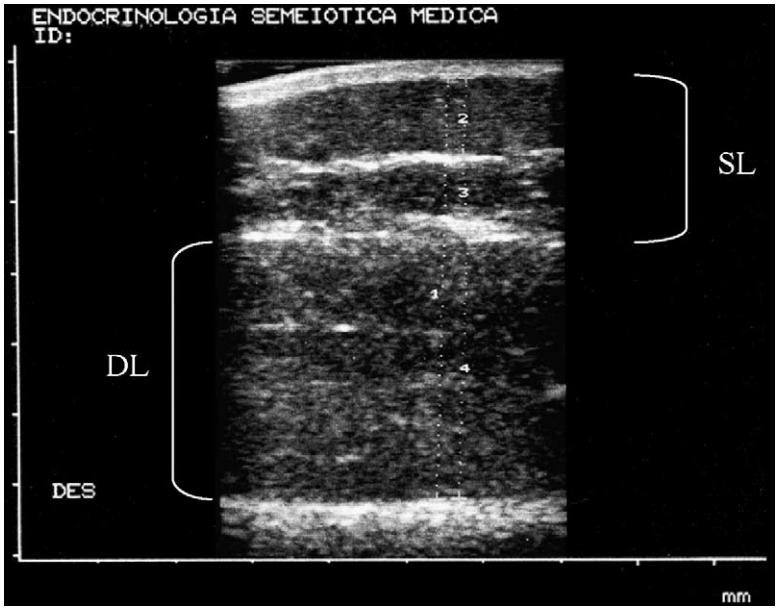

Fig. 1. Ultrasound analysis of thigh fat: two separate layers are evident with dense separating membranes: superficial layer (SL) and the deep layer (DL).

Table 1
Measured parameters in the two groups of subjects ($n = 9$), treated with GA cream or with placebo, before and after treatment

	Treatment with GA cream				Treatment with placebo			
	Treated thigh		Contralateral thigh (untreated)		Treated thigh		Contralateral thigh (untreated)	
	Before	After	Before	After	Before	After	Before	After
Superficial layer (mm)	16.8 ± 1.6[a]	14.7 ± 1.4	16.1 ± 1.5	16.0 ± 1.4	18.3 ± 1.9	17.6 ± 1.7	17.0 ± 1.7	16.3 ± 1.6
Deep layer (mm)	28.8 ± 1.5	28.9 ± 1.2	27.5 ± 1.2	28.2 ± 1.6	33.2 ± 1.4	33.3 ± 1.6	31.8 ± 1.3	32.8 ± 1.5
Circumference at middle of thigh (cm)	52.7 ± 1.0[a]	52.4 ± 1.0	52.9 ± 1.4	52.8 ± 1.4	51.9 ± 1.0	51.8 ± 1.0	51.1 ± 0.9	51.2 ± 0.9
Circumference at top of thigh (cm)	57.4 ± 1.0[a]	57.1 ± 1.0	56.3 ± 1.2	56.4 ± 1.3	56.9 ± 0.9	56.9 ± 0.8	56.5 ± 0.7	56.6 ± 0.6

Data are expressed as mean ± S.E. The ultrasound measurement was performed in triplicate for each subject, and the mean value was considered.
[a] Comparison between treated limb and contralateral, before versus after treatment, $p < 0.005$.

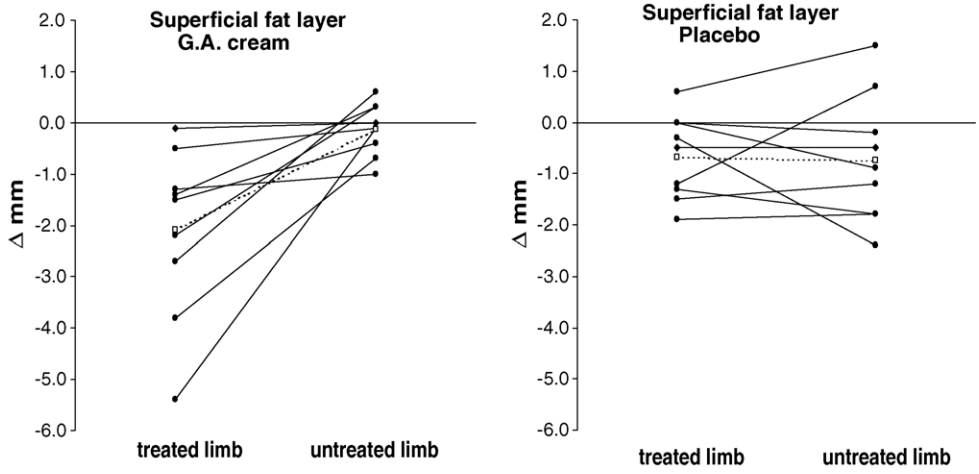

Fig. 2. Absolute variation in thickness of superficial fat layer in patients treated with GA cream (figure on the left) or with placebo (figure on the right). Differences are presented for treated limb and contralateral untreated limb. Dotted lines indicate mean values. Statistical analysis with Wilcoxon Matched-Pairs Signed-Rank Test (two-tailed), comparison between treated limb and untreated limb: for GA cream, $p < 0.02$; for placebo, p is n.s.

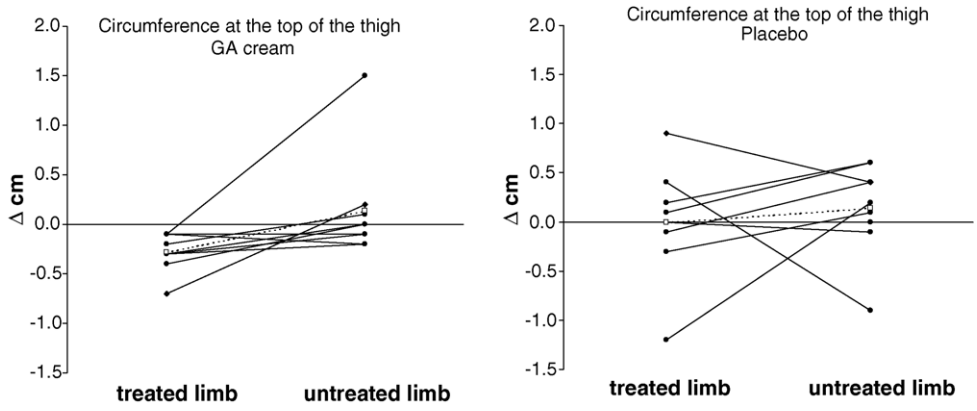

Fig. 3. Absolute variation of circumference at the base of the thigh in patients treated with GA cream (figure on the left) or with placebo (figure on the right). Differences are presented for treated limb and contralateral untreated limb. Dotted lines indicate mean values. Statistical analysis with Wilcoxon Matched-Pairs Signed-Rank Test (two-tailed), comparison between treated limb and untreated limb: for GA cream $p < 0.025$; for placebo, p is n.s.

Table 2
Hormonal parameters before and after treatment with GA and placebo (data are expressed as mean ± S.E.)

	Treatment with G.A. cream		Treatment with placebo cream	
	Before	After	Before	After
Cortisol (μg/dL)	14.4 ± 1.2	13.85 ± 1.3	15.03 ± 1.4	16.28 ± 1.4
Aldosterone (ng/dL)	19.3 ± 1.7	20.6 ± 2.0	17.8 ± 1.2	18.1 ± 1.3
PRA (ng/mL/h)	2.33 ± 0.2	3.03 ± 0.3	2.4 ± 0.2	2.8 ± 0.3

and placebo cream). The results show a clear and significant reduction in the GA-treated subjects but not in the placebo-treated subjects. There was no difference in the thickness of the deep layer in both groups.

A significant difference ($p < 0.005$) was observed considering the net values of the circumference before versus after treatment with GA cream (Table 1) both at the middle of the thigh and at the top ($p < 0.005$). In some cases the treatment with the placebo cream produced an apparent reduction of the circumference but the mean value was not different from pretreatment.

Fig. 3 shows the differences (end of treatment minus before treatment) of the values of the circumferences in the treated and untreated thighs, both with GA cream and placebo cream. The difference was significant only in the GA-treated thigh ($p < 0.025$).

PRA, plasma cortisol and aldosterone were not significantly different from baseline value after either treatment (Table 2).

4. Discussion

The results of the study show that topical application of GA can reduce the thickness of superficial subcutaneous fat and the circumference of the thigh of healthy women with normal BMI. Our data on ultrasound analysis of subcutaneous fat at the level of thigh have confirmed that two fat layers are evident and that GA acts only at the level of the superficial layer. In effect the deep layer did not show any change. We believe that the difference is related to the penetration of GA only at the level of the superficial layer. The lack of effect at the deep layer seems more related to the absence of penetration of GA at that level than to a possible difference in expression of 11HSD1 in the two layers of subcutaneous fat.

It is known that oral licorice intake can produce pseudohyperaldosteronism due to the effect of GA on 11HSD2 [2,6] and on mineralocorticoid receptors [6] and this side effect might be a limitation to the use of a topical cream with GA. The lack of any effect on the renin–aldosterone system was, however, consistent with the fact that topical application of the cream does not produce a significant absorption of GA in the general circulation. The lack of absorption prevents GA from acting on the kidney and other epithelial target tissues, which possess 11HSD2.

We have previously demonstrated that GA also has a direct mineralocorticoid effect by binding to mineralocorticoid receptors [6,7], but in this case the mineralocorticoid effect of GA is not effective since subcutaneous fat is not a target for aldosterone, lacking mineralocorticoid receptors. One possible remark regarding our data is that the lack of general side effects is due to the short-term therapy. We, in effect, treated the subjects only for 1 month, but in previous studies using oral licorice extract we found a clear picture of pseudohyperaldosteronism even after 1 week of treatment [6]. We also found normal plasma concentration of cortisol, and these data are consistent with a local activity of GA without involvement of the liver, which is responsible for the activity of 11HSD1 and cortisol–cortisone metabolism.

Our data are consistent with a block of 11HSD1 at the level of fat tissue. It is known that visceral fat is richer in 11HSD1,

but subcutaneous fat also expresses the enzyme [13]. The block of 11HSD1 reduces the local availability of cortisol for glucocorticoid receptors. It is known that cortisol is involved in the accumulation of triglycerides in adipocytes and in the differentiation of preadipocytes into adipocytes [10,13,14].

GA can also affect fat metabolism and, in particular, the metabolism of cholesterol and ascorbic acid, which are involved in the inactivation of fat lipase and is related to mobilization of fat from adipose stores [15–17]. The reduction of fat was small but significant, and it must be kept in mind that the subjects in this study had a normal BMI. We therefore hypothesize that the effect of the cream may be more evident in subjects with pathological local fat accumulation, considering that in these subjects both the concentration and activity of 11HSD1 are higher. The data from the treated thigh were significantly different from those of the contralateral thigh as well as from those of the subjects treated with placebo. This confirms that the reduction in fat was due to GA and not only to the massage, which was recommended to improve the penetration of the cream.

It is interesting to note that licorice root possesses both estrogen-like and antiestrogen activity due to the presence of different components such as flavonoids and the isoflavan glabridin [18,19]. The use of pure GA has prevented these contrasting effects, which also involve estrogen receptors. Licorice also has an antiandrogen effect by blocking 17 hydroxysteroid dehydrogenase [18,19], but fat tissue does not possess this enzyme which is active at the level of the ovary and adrenals.

The use of a cream, which reduces the superficial fat layer could be additive to the effect of diet and oral anorexic products, especially in those women who have a problem with excessive fat deposition at a specific location like the thigh or abdomen. The general measures to treat overweight women can, in effect, also act at the level of the deep layer of fat.

In conclusion, in a topical application, relatively low amounts of GA can aid in the therapy of obesity and in particular in the local over-accumulation of fat, without affecting the renin–aldosterone system or 11HSD type 2. This preliminary study may serve as the basis of a useful adjunct to diet, and gentler alternative to cosmetic surgery, in people concerned about body shape. Further experiments are necessary in order to confirm these data and to exclude the involvement of other phenomenons.

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