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Effect of licorice on PTH levels in healthy women

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ABSTRACT

Licorice has been considered a medicinal plant for thousands of years. Its most common side effect is hypokalemic hypertension, which is secondary to a block of 11β-hydroxysteroid dehydrogenase type 2 at the level of the kidney, leading to an enhanced mineralocorticoid effect of cortisol. This effect is due to glycyrrhetinic acid, which is the main constituent of the root, but other components are also present, including isoflavans, which have estrogen-like activity, and are thus involved in the modulation of bone metabolism. We investigated nine healthy women 22–26 years old, in the luteal phase of the cycle. They were given 3.5 g of a commercial preparation of licorice (containing 7.6%, w/w of glycyrrhizic acid) daily for 2 months. Plasma renin activity (PRA), aldosterone, cortisol, serum parathyroid hormone (PTH), 1,25-dihydroxy Vitamin D (1,25OHD), 25-hydroxycholecalciferol (25OHD), estradiol, FHS, LH, alkaline phosphatase (ALP), calcium, phosphate and creatinine, urinary calcium and phosphate and mineralometry were measured. PTH, 25OHD and urinary calcium increased significantly from baseline values after 2 months of therapy, while 1,25OHD and ALP did not change during treatment. All these parameters returned to pretreatment levels 1 month after discontinuation of licorice. PRA and aldosterone were depressed during therapy, while blood pressure and plasma cortisol remained unchanged. *Conclusions*: licorice can increase serum PTH and urinary calcium levels from baseline value in healthy women after only 2 months of treatment. The effect of licorice on calcium metabolism is probably influenced by several components of the root, which show aldosterone-like, estrogen-like and antiandrogen activity.

1. Introduction

Bone metabolism and PTH are affected by the activity of different classes of steroids. Androgens inhibit osteoclast formation induced by parathyroid hormone (PTH), an effect which is androgen receptor-mediated [1]. In primary aldosteronism (PA), higher PTH concentrations are needed to maintain low-normal levels of ionized calcium [2]. In addition, in patients with PA, 1,25-dihydroxy Vitamin D (1,25OHD) is significantly lower than in those with low-renin essential hypertension, suggesting that mineralocorticoids can exert primary effects on the renal production of 1,25OHD and account for the abnormal relation of 1,25OHD to PTH [3].

In the literature, there is no report on the effect of licorice on PTH and calcium homeostasis. The most common side effect of the chronic consumption of licorice in high amounts is an hypokalemic hypertension, due to the block of the activity of 11β-hydroxysteroid dehydrogenase type 2 (11HSD2) by glycyrrhetinic acid (GA) at the level of the kidney and other aldosterone targets [4,5]. In these tissues, 11HSD2 plays a key role in modulating mineralocorticoid effects in classical target tissues of aldosterone by inactivating cortisol to cortisone [4–6]. In addition, GA can bind directly to mineralocorticoid

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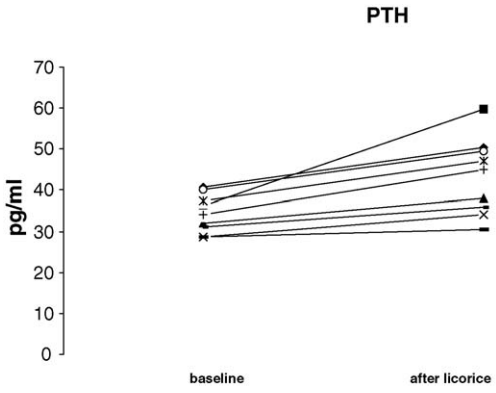

Fig. 1 – PTH values before and after treatment with licorice in the nine subjects.

receptors as an agonist when its plasma concentration is high enough to compete with aldosterone and cortisol for mineralocorticoid receptors [6–8]. The plasma concentration of GA, after ingestion of 225 mg of glycyrrhizic acid, is 10 mg/dl [9], and its affinity for mineralocorticoid receptors is 1:3000 that of aldosterone [7,8]; although GA is extensively bound to plasma albumin the free amount can be high enough to have a direct mineralocorticoid activity [9]. GA is also a potent inhibitor of 11β-hydroxysteroid-dehydrogenase type 1 (11HSD1), which converts cortisone to cortisol (Fig. 1), and therefore regulates the availability of cortisol at the level of liver, fat and bone [10]. The reduced local availability of cortisol due to licorice could have beneficial effects by preventing the osteoporotic effects of excess glucocorticoids [11]. GA does not bind to estrogen or to androgen receptors [7].

Licorice has been used as a medicinal plant for thousands of year [12]. The active component of licorice, glycyrrhizic acid, is completely hydrolyzed in vivo into GA [9], which is responsible for most of its pharmacological properties. We have previously demonstrated that after 1 week of licorice intake total serum testosterone is slightly but significantly reduced in healthy males and females [13,14]. The inhibitory effect in vitro of GA (10 μg/ml) on 17 beta-hydroxysteroid dehydrogenase (17HSD) is 90%, with a median effective dose of 4 μM [15]. These properties of GA are consistent with an in vivo effect after administration of high amounts of licorice. This property of licorice can inhibit the conversion of androstenedione into testosterone. GA can also and stimulate the aromatization of testosterone to estradiol, at a concentration of 0.3 mM in the incubation medium in vitro [15], and also blocks the activity of 3-β-hydroxysteroid dehydrogenase (3HSD) and 17–20 lyase [13–15,19]. At 10 mM GA and can affect the androgen metabolism also in vivo. All these enzymes are involved in the synthesis and/or metabolism of androgens and estrogens.

Acetone or ethanol extracts of licorice root also contains subclasses of the flavonoid family (isoflavans and isoflavones) and chalcones (glabridrin, glabrol, glabrene, 3-hydroxyglabrol, 4′O-methylglabridin hispaglabridin A and hispaglabridin B) [16]. Glabridin is present in the extract at more than 11% (w/w) [17] and exhibits diverse biological activities, including estrogen-like properties, with affinity for estrogen receptors 10^4 lower than that of estradiol [16,18]. Other components of the root with estrogen-like activity, such as glabrene and genistein, can bind to the estrogen receptor with the same affinity as glabridin [16]. Although these components are found in extracts of root of licorice at very low concentrations (0.2–2%, w/w) they may be able to modulate bone metabolism in post-menopausal women [17].

The aim of this study was thus to evaluate the effect of licorice on mineral metabolism in healthy women, given that chronic licorice consumption may directly or indirectly produce many hormonal effects.

2. Experimental

Nine healthy women aged 22–26 years from the staff of the Department of Medical and Surgical Science of the University of Padua were enrolled for this study. All the volunteers were informed about the study and gave their consent. The protocol was conducted over spring and was approved by the local ethics committee. The subjects had normal BMI (23.4 ± 2.1), were regularly menstruating, and did not take contraceptives or other substances that could interfere with hormonal measurements. None of the volunteers showed clinical, ultrasonographic or laboratory signs of either hyperandrogenism or polycystic ovary syndrome. They consumed, in fractionated doses, 3.5 g a day of a commercial preparation of pure licorice in the form of tablets (Saila, Italy), containing 7.6% (w/w) of glycyrrhizic acid, as determined by mass spectrometry–gas chromatography [20]. The calculated daily amount of glycyrrhizic acid was 266 mg. The treatment lasted for two menstrual cycles (56 days) so that all the measurements could be taken in the luteal phase. Before treatment, during treatment (in the luteal phase of the two cycles), and after 28 days wash-out, the following parameters were evaluated: PTH, 1,25OHD, 25-hydroxycholecalciferol (25OHD), plasma renin activity (PRA), cortisol, aldosterone, estradiol, FSH, LH, testosterone and sex steroid binding globulin (SHBG). Systolic and diastolic blood pressures were also measured in the sitting position (mean of three measurements). Commercial radioimmunoassay kits (Medical System, Genova, Italy) were used to measure the plasma hormonal parameters and SHBG (variation coefficients: inter-assay lower than 11% and intra-assay lower than 9%). Intact PTH, 1,25OHD and 25OHD were measured in serum by two-site immunoradiometric assay (Biochem Immunosystem Italia, Bologna, Italy). A concentration of 500,000 pg/ml of PTH [1–34], PTH [44–68] and PTH [53–84] showed no measurable cross-reactivity. Alkaline phosphatase activity (ALP), serum calcium, serum phosphate, plasma creatinine, urinary calcium and urinary phosphate were assessed by common laboratory methods.

Results are expressed as the mean ± standard deviation from the mean. The statistical evaluation was performed by Wilcoxon matched-pairs signed ranks test. A p-value of less than 0.05 was considered statistically significant.

3. Results

The values of the parameters investigated for each subject are shown in Figs. 1 and 2, and the mean ± S.D. is reported in Table 1.

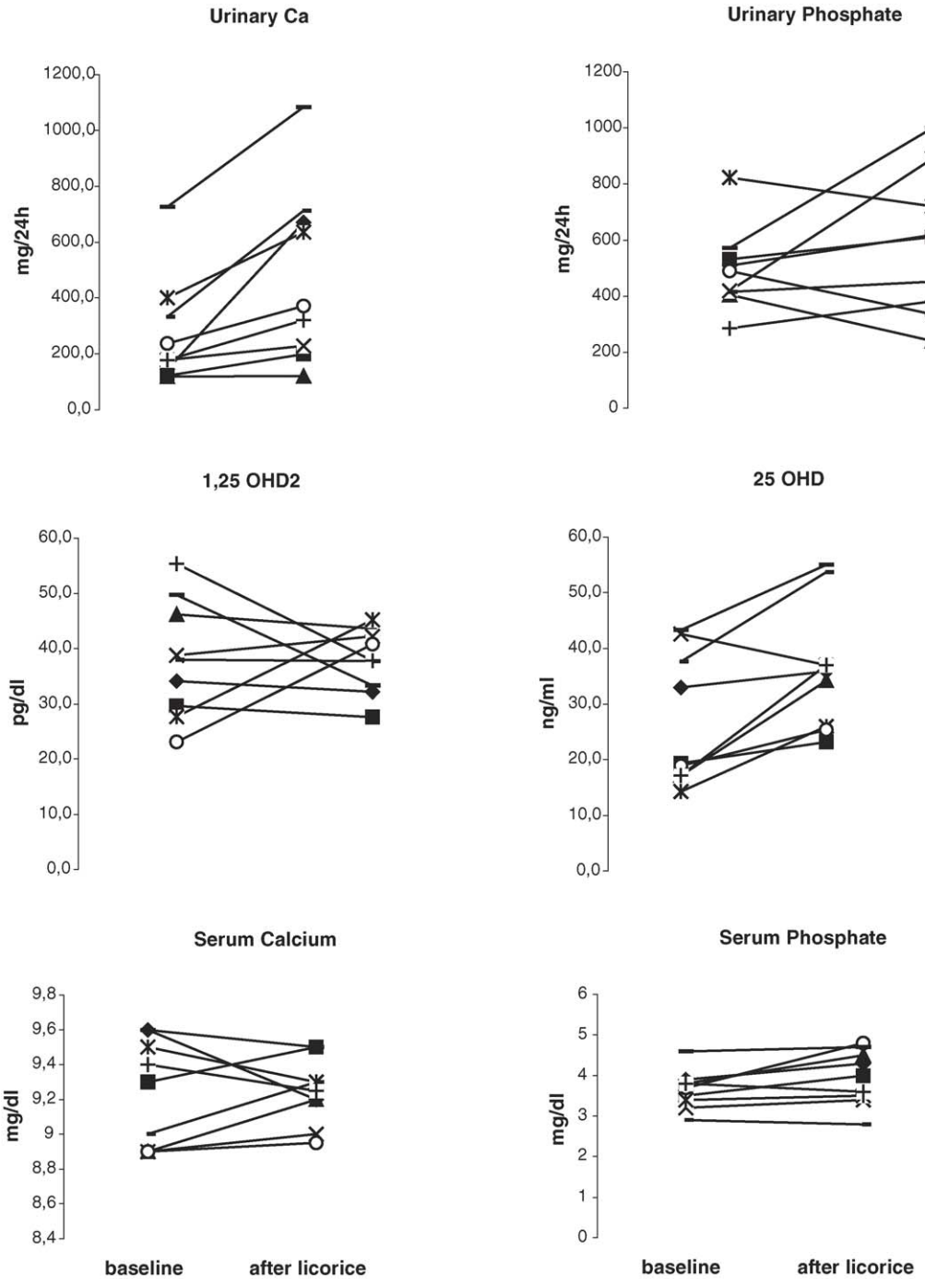

Fig. 2 – Urinary calcium and phosphate, 1,25OHD, 25OHD, serum calcium and phosphate in the nine subjects, before, during and after licorice.

PTH, urinary calcium and 25OHD showed a slight but significant increase after two cycles of therapy [PTH: baseline 36.0 ± 5.5; after two cycles 43.3 ± 9.5 ($p = 0.003$); urinary calcium: baseline 270 ± 180; after two cycles, 482 ± 346 mg/24 h ($p < 0.003$); 25OHD: basal 27.3 ± 11.3; after two cycles, 36.3 ± 12.3 ($p < 0.01$)]. An increase of PTH was evident in all the subjects (Fig. 1). The values of PTH returned to pretreatment level after withdrawal, while 25OHD remained elevated versus pretreatment ($p < 0.01$). Vitamin D 1-25, ALP, serum calcium, serum phosphate and creatinine were unchanged at the end of treatment (Table 1 and Fig. 2). The mean diastolic and systolic blood pressures were not modified by the treatment (before treatment, $110.5 \pm 9.7/70.0 \pm 8.8$ mmHg; after two cycles, $116.9 \pm 9.2/73.1 \pm 10.3$ mmHg and after discontinuation, $112.7 \pm 6.5/69.4 \pm 7.3$ mmHg). The data on serum testosterone, PRA and plasma aldosterone have already been reported [13] and have shown that all these steroids decrease significantly after licorice treatment. FSH, LH, estradiol and

Table 1 – Mean ± S.D. of hormonal parameters and blood pressure in nine female subjects during licorice consumption and after wash-out

	Before	First cycle	Second cycle	Wash-out
PTH (pg/ml)	36.0 ± 5.5	40.8 ± 15.3	43.3 ± 9.5*	38.5 ± 9.2
1,25OHD (pg/ml)	38.0 ± 12.4	42.7 ± 8.2	37.8 ± 6.2	39.2 ± 11.8
25OHD (ng/ml)	27.3 ± 11.3	31.2 ± 12.0	36.3 ± 12.3**	39.3 ± 11.7**
Serum Ca (mg/dl)	9.2 ± 0.3	9.3 ± 0.3	9.2 ± 0.2	9.3 ± 0.3
Serum P (mg/dl)	3.6 ± 0.4	4.2 ± 0.3	4.0 ± 0.4	3.9 ± 0.6
Urinary Ca (mg/24 h)	270 ± 180	308 ± 214	482 ± 346*	348 ± 290
Urinary P (mg/24 h)	494 ± 187	545 ± 259	583 ± 374	510 ± 355
ALP (U/l)	51.9 ± 12.6	46.8 ± 14.6	45.0 ± 9.6	48.5 ± 9.5
PRA (ng/ml/h)	3.1 ± 1.2	1.4 ± 0.7***	1.1 ± 0.7***	0.5 ± 0.4***
Up–right plasma aldosterone (ng/dl)	14.5 ± 6.3	6.5 ± 3.8***	5.6 ± 3.5***	14.7 ± 10.0
Plasma cortisol (μg/dl)	16.1 ± 3.7	17.9 ± 3.2	18.6 ± 3.4	16.6 ± 3.5
Total testosterone (ng/dl)	27.8 ± 8.2	19.0 ± 9.4**	17.5 ± 6.4**	27.0 ± 13.8
SHBG (nmol/l)	34 ± 21	29 ± 19	27 ± 17	29 ± 19
FSH (ng/ml)	4.2 ± 2.3	–	5.2 ± 2.2	4.9 ± 2.3
LH (ng/ml)	4.8 ± 2.3	–	4.6 ± 2.8	3.5 ± 2.5
Estradiol (pg/ml)	170 ± 32	–	180 ± 48	189 ± 57
Systolic BP (mmHg)	110.5 ± 9.7	114.4 ± 10.7	116.9 ± 9.2	112.7 ± 6.5
Diastolic BP (mmHg)	70.0 ± 8.8	73.9 ± 11.1	73.1 ± 10.3	69.4 ± 7.3

* $p < 0.05$ vs. before.
** $p < 0.01$ vs. before.
*** $p < 0.001$ vs. before.

cortisol values were not modified by licorice treatment (Table 1).

4. Discussion

Our results show a slight increase in PTH and in urinary calcium from baseline values after the short-term administration of licorice in healthy women. Because the measurements were all performed in the same phase of the cycle, an influence of sex hormone variation can be ruled out. The compliance of the subjects with the treatment is in addition demonstrated by the suppression of PRA and aldosterone. It is worth noting that the values of PRA were suppressed even after 1-month withdrawal of licorice while plasma aldosterone returned to pretreatment. The possible explanation for this finding is that the renin-angiotensin system needs more time to be reactivated after prolonged suppression due to volume expansion. The normal levels of plasma aldosterone could be consistent with temporarily increased sensitivity of athe drenal glomerulosa to angiotensin II after suspension of licorice.

4.1. *Implication of mineralocorticoid-like properties of licorice*

A simple interpretation of our data is that GA can affect PTH concentration due to its mineralocorticoid-like properties. It is known that licorice can produce pseudohyperaldosteronism, and an increase of PTH and inverse correlation between PTH and 1,25OHD have been reported in patients with PA [2,3]. In PA, the increase of PTH counteracts the decrease of ionized calcium, due to the direct effect of aldosterone excess at the kidney level or to decrease of 1,25OHD. In any case the increase of PTH is not accompanied with an increased calcium excretion in these patients.

It is difficult to believe that in our subjects a very small increase of PTH can produce calcium excretion, since physiological concentrations of PTH usually reduce the excretion of calcium, while increase in calcium excretion is peculiar to primary hyperparathyroidism, when the ability of the kidney to reabsorb calcium is overcome. A slight increase of PTH could indeed be beneficial if we consider that physiological concentrations of PTH are needed for a correct bone metabolism. Administration of low doses of PTH has an anabolic effect at bone in animals and humans, whereas high doses, administered continuously are associated with catabolic effects. In both women and men PTH leads to an increase in bone density in doses that do not regularly lead to adverse events like hypercalcemia [18]. We did not find any change in serum calcium, 1,25OHD and bone density (data not shown) during treatment. It is therefore more likely that the increased excretion of calcium versus pretreatment can be really related to the direct (via mineralocorticoid receptors) and/or indirect (via 11HSD2 block) mineralocorticoid activity of licorice. The discrepancy between our data and those reported for PA is probably due to the fact that our subjects were consuming licorice for only 2 months, while PA normally has a much longer duration. Our patients had a normal blood pressure and serum potassium after therapy and maybe more time is necessary to show the full electrolyte and metabolic picture. Furthermore, the reduced availability of cortisol at the level of bone, due to the block of 11HSD1, can prevent a local loss of calcium. This effect might counteract the increased excretion of calcium due to the effect of GA on 11HSD2 in kidney.

4.2. *Implications of the estrogenic and antiandrogenic properties of licorice*

The possible involvement of other factors must also be considered in the interpretation of our results. Licorice exhibits

many other hormonal effects in addition to those via mineralocorticoid receptors. It is known that licorice root is made up of different compounds, the most important of them being GA and other flavanoids [17]. The root extract has an antiandrogen property (due to GA) and an estrogen-like (due to glabridin, which is contained in high amounts in the root extract) [17,19,21–23]. It is known that androgens play an important role in the regulation of bone metabolism in humans, as androgen treatment is effective in increasing bone mineral density in osteoporosis [1,24]. Testosterone also acts directly on osteoblasts, stimulating the growth and differentiation of osteoblasts in vitro by binding to androgen receptors [1]. Licorice was able to decrease significantly the values of serum testosterone in our subjects, but the value was still within the normal range and therefore the implication of the decrease of testosterone is unlikely to be responsible for our findings.

More importance should be given to the action of isoflavonoids (glabridin and glabrene) with estrogen-like activity, which could have a beneficial effect on calcium homeostasis, as previously reported by Tamir et al. [19]. The affinity of these compounds for estrogen receptors is very low, but considering the amount of licorice ingested a positive effect could be observed by the estrogenic activity of isoflavonoids and in particular of glabridin, concentrations of which are even higher than that of GA [19]. In vitro studies have documented that glabridin increases the function of osteoblastic cells [17]. It is known that estrogens can also increase PTH secretion and it is possible that phytoestrogens are also able to produce such an effect [25], consistent with our results. A study done in premenopausal women shows a correlation between PTH values and the amount of isoflavone consumed. Finally, we were not able to observe any modification of FSH during treatment with licorice but this cannot exclude an additional selective estrogenic effect of licorice at the level of bone, since the recent studies using synthetic estrogens have clearly identified selective tissue estrogenic activity regulators (STEAR) [26].

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