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Licorice Consumption and Serum Testosterone in Healthy Man

Abstract

We have previously found that licorice can reduce serum testosterone in healthy men. These results were not confirmed in another study, where the same amounts of licorice did not decrease salivary testosterone values. In the actual study we treated more cases with the same amount of licorice and reproduced our previous data. The mean testosterone values decreased by 26% after one week of treatment ($p < 0.01$). There was also a significant increase in 17-OHP and LH concentrations and a slight, but not significant decrease in free testosterone. Licorice treatment, in addition, did not affect the response of testosterone and 17-OHP to stimulation with β-HCG.

Key words
Licorice · glycyrrhetinic acid · total testosterone · free testosterone

Introduction

Licorice roots and their extracts have been used since over one thousand years as medical herb products and as sweeteners and mouth refreshers (Armanini et al., 2002). The active principle of licorice is glycyrrhizic acid, which is hydrolyzed into its aglicone glycyrrhetinic acid *in vivo*. The most widely known side effect of chronic ingestion of the compound is the syndrome of pseudohyperaldosteronism, which is characterized by hypertension, hypokalemia, metabolic alkalosis, low plasma renin activity (PRA) and aldosterone, and an increase in the cortisol/cortisone ratio (F/E) in urine (Farese et al., 1991; Armanini et al., 1996).

Pseudohyperaldosteronism is due to block of type 2 11β-hydroxy-steroid dehydrogenase (Funder et al., 1988; Stewart and Krozowski, 1999) (11HSD2) and to the binding of glycyrrhetinic acid to mineralocorticoid receptors, when its plasma concentration becomes high enough to bind to the receptor (Armanini et al., 1996). The block of 11HSD2, by glycyrrhetinic acid, prevents the conversion of cortisol to its inactive metabolite cortisone (Armanini et al., 1996; Funder et al., 1988; Stewart and Krozowski, 1999) and, as a consequence, cortisol can bind to mineralocorticoid receptors and produce an acquired syndrome of apparent mineralocorticoid excess.

Licorice root possesses many other endocrine properties, which are related both to its active principle, glycyrrhetinic acid, and to other components of the root (Armanini et al., 2002). We have previously described a significant reduction in total serum testosterone and an increase in 17-hydroxyprogesterone (17-OHP) in a group of 7 healthy volunteers, who consumed 7 grams of a commercial preparation of licorice per day (Armanini et al., 1999). These findings are consistent with *in vivo* and *in vitro* studies, which have found that the active principle of licorice, glycyrrhetinic acid, inhibits the activity of 17-hyhdroxysteroid dehydrogenase (17-HSD) and of 17–20 lyase. 17 HSD catalyzes the conversion of androstenedione to testosterone, while 17–20 lyase converts 17-OHP into androstenedione (Sakamoto et al., 1988; Yaginuma et al., 1982; Takeuchi et al., 1991).

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Received: February 10, 2003 · **First decision:** March 17, 2003 · **Accepted:** March 22, 2003

Bibliography
Exp Clin Endocrinol Diabetes 2003; 111: 341–343 © J. A. Barth Verlag in Georg Thieme Verlag KG · Stuttgart · New York · ISSN 0947-7349

Josephs and colleagues have recently reported a small but not significant reduction in salivary testosterone in healthy volunteers, after 4 days consumption of the same amount of a glycyrrhizic acid as our cases (Josephs et al., 2001). The aim of our study was to explain the different results obtained in these two experimental studies.

Subjects and Methods

Seventeen healthy male students (age range: 22 – 24 years) were recruited for the study. All the volunteers were fully informed about the experiment and gave their informed consent. The protocol was approved by our ethical committee. They consumed 7 grams a day of a commercial preparation of licorice in the form of tablets (Saila Lainate, Italy), containing 7.6% W.W. of glycyrrhizic acid, as determined by mass spectrometry-gas chromatography (Bernardi et al., 1994). The treatment was continued for 7 days with measurement at day 4 and 8 of serum or plasma hormonal parameters (LH, PRA, aldosterone, cortisol, total and free testosterone, androstenedione, 17-OHP) and of the concentration of cortisol and cortisone in urine. In 11 cases the same hematological parameters were measured after 3 days of withdrawal of licorice. In the other 6 cases, the consumption was prolonged for 4 more days, and at the 1st and 3rd days, 2000 IU of β–chronic gonadotrophin (βHCG) were injected, to measure total and free testosterone, androstenedione and 17-OHP, the day following each injection. A control group of 6 male volunteers of the same age were treated with a black placebo and underwent the same stimulation test.

Serum free and total testosterone, androstenedione, luteinizing hormone (LH), 17-OHP, plasma aldosterone and PRA were measured by immunoradioassay commercial kits (variation coefficient: inter-assay lower that 11% and intra-assay lower than 9%).

The concentration of cortisol and cortisone in urine was measured by gas chromatography-mass spectrometry, as previously described (Palermo et al., 1996).

The statistical evaluation of parameters against pre-treatment values was done by repeated measure analysis of variance with post hoc Bonferroni. For statistical evaluation of % increase of hormonal parameters after β-HCG injection, the χ^2 test was used. The level of statistical significance was considered to be $p < 0.05$.

Results

We found (Table **1**) a reduction of 25% in serum testosterone, during licorice consumption ($p < 0.05$), at both days 4 and 8 (21.0 ± 7.0 nmol/l pretreatment, 15.9 ± 5.1 nmol/l at day 4 and 15.8 ± 5.7 nmol/l at day 8), and an increase of 39% in 17-OHP at day 8 (2.8 ± 1.2 nmol/l pretreatment, 3.9 ± 2.1 nmol/l, $p < 0.05$). The values of PRA and aldosterone were suppressed by licorice ($p < 0.01$), while the cortisol/cortisone ratio in urine was significantly increased (from 0.97 ± 0.42 to 1.44 ± 0.71).

Serum androstenedione values were not modified by the therapy (5.0 ± 0.5 nmol/l pretreatment, 4.3 ± 0.3 nmol/l at day 4 and 4.9 ± 1.8 nmol/l at day 8). Serum LH showed a significant increase at day 8 (from 3.1 to 4.0 IU/L, $p < 0.05$).

Table **1** Mean ± SD of serum hormone concentrations in seventeen men before (day 0) and during licorice consumption (day 4 and day 8)

	Day 0	*Day 4*	*Day 8*
Total testosterone (nmol/L)	21.0 ± 7.0	15.9 ± 5.1**	15.8 ± 5.7**
Free testosterone (pmol/L)	17.6 + 6.1	16.4 + 7.0	16.5 + 6.8
Androstenedione (nmol/L)	5.0 ± 1.75	4.3 ± 1.1	4.9 ± 1.8
17OH-progesterone (nmol/L)	2.8 ± 1.2	3.2 ± 2.0	3.9 ± 2.1*
LH (IU/L)	3.1 ± 1.1	3.3 ± 1.3	4.0 ± 0.8*
Plasma aldosterone (nmol/L)	0.38 ± 0.09	0.14 ± 0.04**	0.09 ± 0.02**
PRA (ng/ml/h)	4.5 ± 1.7	1.7 ± 1.3**	1.1 ± 1.0**
Urinary F/E	0.97 ± 0.42	–	1.44 ± 0.71*

* $p < 0.05$ *vs* day 0, ** $p < 0.01$ *vs* day 0

In the eleven cases who were studied three days after suspension, mean serum free and total testosterone returned to pretreatment values (total testosterone pretreatment 21.2 ± 7.9, after withdrawal to 20.2 ± 6.1 nmol/l; free testosterone 17.4 ± 6.0 to 16.5 ± 6.2 pmol/l, $p > 0.05$).

Plasma aldosterone was still reduced (from 0.36 ± 0.5 to 0.10 ± 0.07 nmol/l; $p < 0.001$).

In the six cases who underwent βHCG stimulation, serum total testosterone decreased in all the cases at day 8 of licorice alone (mean decrease of 25% +, $p < 0.05$). The % increase of total and free testosterone, after the two β-HCG injections, was similar ($p > 0.05$) in both licorice-treated and placebo-treated subjects (total testosterone in licorice treated: 90% after the first and 107% after the second injection of β-HCG; in placebo treated: 82% and 105%; free testosterone in licorice treated 70% and 70%, placebo-treated 102% and 109%).

The values of 17-OHP after β-HCG injection increased by 59% and 37% in licorice treated and by 96% and 44% in placebo treated subjects ($p > 0.05$).

Serum androstenedione was not significantly affected by stimulation with β-HCG.

None of the patients had values of total and free testosterone lower than normal range during therapy, and none complained of unwanted sexual effects.

Blood pressure was not affected by the treatment (pre-treatment systolic 121 ± 5.2, diastolic 83 ± 5.0 mm Hg; during licorice systolic 125 ± 7.8, diastolic 84 ± 5.9 mm Hg).

Discussion

The results obtained confirm those of our preliminary study, which demonstrated a significant decrease in serum total testosterone during licorice consumption (Armanini et al., 1999). Another recent study reported unchanged values of salivary testosterone using the same protocol as ours (Takeuchi et al., 1991). These authors measured salivary testosterone, claiming that the values of total serum testosterone parallelled those of salivary testosterone. Salivary testosterone is mainly unbound and a comparison between the two measurements can be done in healthy subjects free of therapy, but not during consumption of licorice, whose active metabolite, glycyrrhetinic acid, could bind to SHBG (Tamaya et al., 1986). We also found an increase in 17-OHP, without any change in androstenedione in agreement with an effect of licorice on 17 – 20 lyase. *In vitro* studies have, in effect, shown that incubation of Leydig cells with glycyrrhetinic acid produces a significant decrease in testosterone production and an accumulation of 17-OHP (Sakamoto and Wakabayashi, 1988). The effect of licorice on 17 HSD and 17 – 20 lyase is weak and transient, since it was reversed after 4 days suspension or by stimulation of testosterone with βHCG. Indeed, the effect of glycyrrhetinic acid on 11HSD2 and on Type I corticosteroid receptors is still evident 4 days following the withdrawal of licorice (Armanini et al., 1996).

It is worthy of note that serum free testosterone was only slightly reduced in both our study and in the study by Josephs. These data can be considered consistent with a complex series of direct and compensatory events, which follow the ingestion of licorice. It is known that the licorice root possesses many endocrine-like properties, thus influencing other hormone parameters. If we consider only the effect on androgen metabolism, the initial event is an inhibition by glycyrrhetinic acid of the synthesis of testosterone and androstenedione. We found no change in serum androstenedione, probably due to the concomitant block of 17 – 20 lyase, as demonstrated by the increase in 17-OHP. The decrease in total testosterone, due to the enzymatic block, stimulates the hypothalamic-pituitary axis to produce LH. The effect of LH on Leydig cells could, after some days, override the enzymatic block of testosterone synthesis and restore a normal concentration of free testosterone. This interpretation is consistent with our results and with those of Josephs. The increase in the serum glycyrrhetinic acid concentration can progressively lead to a competition of glycyrrhetinic acid and endogenous testosterone for sex steroid-binding protein and initially render the effect of licorice in reducing total testosterone synthesis at the gonadic level more evident.

Another factor which might be involved is the effect of licorice on type 1 11HSD at the level of Leydig cells (Wang et al., 2002; Ge et al., 1996; Leckie et al., 1998). It has been demonstrated that glycyrrhizic acid administration can reduce testosterone synthesis when incubated in the presence of corticosterone or cortisol (Monder et al., 1994; Hardy and Ganjam, 1997). Finally, the aim of future studies will be to use a pharmacological preparation of glycyrrhetinic acid but not a commercial preparation of the root in order to avoid the interference of other substances contained in this root.

Acknowledgements

The authors wish to thank Dr. Edoardo Casiglia for expert assistance with statistical analysis of the data.

References

[1] Armanini D, Bonanni G, Palermo M. Reduction of serum testosterone in men by licorice. N Engl J Med 1999; 341: 1158

[2] Armanini D, Fiore C, Mattarello MJ, Bielenberg J, Palermo M. History of the endocrine effects of licorice. Exp Clin Endocrinol Diabetes 2002; 110: 1 – 5

[3] Armanini D, Lewicka S, Pratesi C, Scali M, Zennaro MC, Zovato S, Gottardo C, Simoncini M, Spigariol A, Zampollo V. Further studies on the mechanism of the mineralocorticoid action of licorice in humans. J Endocrinol Invest 1996; 19: 624 – 629

[4] Bernardi M, D'Intino P, Trevisani F, Cantelli-Forti GI, Raggi MA, Turchetto E, Gasbarrini G. Effects of prolonged ingestion of graded doses of licorice by healthy volunteers. Life Science 1994; 55: 863

[5] Farese RV, Biglieri EG, Shackleton CHL, Irony I, Gomez-Fontes R. Licorice-induced hypermineralocorticoidism. New Engl J Med 1991; 325: 1225 – 1227

[6] Funder JW, Pearce PT, Smith R, Smith A. Mineralocorticoid action: target tissue specificity is enzyme not receptor mediated. Science 1988; 242: 583 – 585

[7] Ge RS, Gao HB, Nacharaju VL, Gunsalus GL, Hardy MP. Identification of a kinetically distinct activity of 11β- hydroxysteroid dehydrogenase in rat Leydig cells. Endocrinology 1996; 138: 2435 – 2442

[8] Hardy MP, Ganjam VK. Stress, 11 beta-HSD, and Leydig cell function. J Androl 1997; 18: 475 – 479

[9] Josephs RA, Guinn JS, Harper ML, Askari F. Liquorice consumption and salivary testosterone concentrations. Lancet 2001; 358: 1613 – 1614

[10] Leckie CM, Wellberg LA, Seckl JR. 11 beta-hydroxysteroid dehydrogenase is a predominant reductase in intact rat Leydig cells. J Endocrinol 1998; 159: 233 – 238

[11] Monder C, Hardy MP, Blanchard RJ, Blanchard DC. Comparative aspects of 11β-hydroxysteroid dehydrogenase. Testicular 11βhydroxysteroid-dehydrogenase. Development of a model for the mediation of Leydig cells function by corticosteroids. Steroids 1994; 59: 69 – 73

[12] Palermo M, Gomez-Sanchez C, Roitman E, Schackleton CHL. Quantitation of cortisol 3-oxo-4-ene steroids in urine using gas chromatography/mass spectrometry with stable isotope-labeled internal standars. Steroids 1996; 61: 583 – 589

[13] Sakamoto K, Wakabayashi K. Inhibitory effect of glycyrrhetinic acid on testosterone production in rat gonads. Endocrinol Jpn 1988; 35: 333 – 342

[14] Stewart PM, Krozowski ZS. 11 Beta-hydroxysteroid dehydrogenase. Vitam Horm 1999; 57: 249 – 324

[15] Takeuchi T, Nishii O, Okamura T, Yaginuma T. Effect of paeoniflorin, glycyrrhizin and glycyrrhetinic acid on ovarian androgen production. Am J Chinese Med 1991; 19: 73 – 78

[16] Tamaya T, Sato S, Okada H. Possible mechanisms of steroid action of the plant herb extracts glycyrrhizin, glycyrrhetinic acid, and paeoniflorin: inhibition by plant herb extracts of steroid protein binding in the rabbit. Am J Obstet Gynecol 1986; 155: 1134 – 1139

[17] Wang GM, Ge RS, Latif SA, Morris DJ, Hardy MP. Expression of 11 beta-hydroxylase in rat Leydig cells. Endocrinology 2002; 143: 621 – 626

[18] Yaginuma T, Izumi R, Yasui H, Arai T, Kawabata M. Effect of traditional herbal medicine on serum testosterone levels and its induction of regular ovulation in hyperandrogenic and oligomenorrheic women. Acta Obst Gynaec Jpn 1982; 34: 939 – 944