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ORIGINAL ARTICLE

Effects of standardized *Ginkgo biloba* extract complexed with phosphatidylserine (Virtiva®) on physiological response to prolonged, intense physical activity

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ABSTRACT

BACKGROUND: Previous studies demonstrated that administration of standardized extract of *Ginkgo biloba* reduces stress-induced cortisol increase in young healthy individuals. This study investigates the effect of chronic and acute administration of a standardized extract of *Ginkgo biloba* (GBE) complexed with phosphatidylserine (Virtiva®) on salivary cortisol level and body composition in elite female volleyball players.

METHODS: Ten elite female volleyball players were randomly assigned to Virtiva® or placebo capsules for 8-week. Moreover, 6 female volleyball players were involved in an acute administration study consisting in 7 days of treatment followed by a 7-day wash-out. Concentration of salivary cortisol, body weight and body composition parameters were measured.

RESULTS: Virtiva®-treated athletes showed a significantly lower salivary cortisol level compared with placebo recipients. Furthermore, Virtiva® administration was associated with a significantly greater increase of fat-free mass and with a significantly greater decrease in fat mass. No significant difference was observed on salivary cortisol level with the acute administration of Virtiva®.

CONCLUSIONS: Our study suggests that administration of standardized GBE combined with phosphatidylserine (Virtiva®) might exert a beneficial effect on athletes during prolonged intensive training programs, by reducing stress induced-cortisol release.

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Ginkgo biloba, also known as Maidenhair tree, is one of the oldest tree species in the world. *Ginkgo biloba* leaves have been largely used in China and Japan as a traditional medicine for a range of conditions, including asthma, bronchitis, cough, and enuresis, for at least 5000 years. The major bioactive components of *Ginkgo biloba* are flavonoids (ginkgo-flavone glycosides) and terpenoids (ginkgolides and bilobalide).[1] The beneficial effects of *Ginkgo biloba* leaves may be associated with a single active ingredient or with the combined action of the multiple bioactive agents found in these plants.

Modern research has focused its interest on the *Ginkgo biloba* leaves extract (GBE) with standardized content of the primary active

components, namely the flavone glycosides (titrated as 24%) and terpenoids (titrated as 6%). Nowadays, GBE represents one of the most popular medicinal plant derivative to alleviate symptoms associated with a range of cognitive disorders such as dementia and age-related memory impairment.[2-6] Chronic administration of GBE has also shown to be effective in cognitive declines associated with ageing and dementia as well as cognitive performance in both healthy older and young adults.[7-10]

An important limiting factor related to the GBE is the reduced bioavailability of active flavonoids due to extensive metabolism following oral administration. The combination with phospholipids was proven to be effective in improving GBE effectiveness in cerebral function. However, it is still unknown if this beneficial effect is due to the mere combination that increases GBE active components availability, or to the synergic psycho-pharmacological actions of the two components.[11]

GBE was also found to exert a salutary effect on mood.[12] In fact, Jezova *et al.* demonstrated that standardized GBE has an inhibitory action on blood pressure and may influence cortisol release in response to a combined stress stimulus consisting of mental load and static exercise.[12] In the sport setting, athletes are constantly submitted to a wide range of stressors, at both physiological and psychological level. Studies have shown that salivary steroid hormones, such as cortisol, are more sensitive to sports induced-stress than other hormones.[13-15] Cortisol, also known as the stress hormone, is a steroid hormone produced by the adrenal cortex that is essential to the maintenance of body homeostasis. It plays a role in blood pressure control, immune system function and metabolism of fats, carbohydrates, and protein. However, the role of GBE on cortisol levels — and consequently on body composition and metabolisms — needs further investigation.

This study evaluates the effect of chronic and acute administration of a standardized GBE complexed with phosphatidylserine (Virtiva®) on salivary cortisol level and body composition in elite female volleyball players during the competition season.

Materials and methods

Subjects and procedures

This study was composed of two parts. The first — chronic part — was a randomized, double-blind, placebo-controlled trial that involved 10 elite female volleyball players playing in the Italian volleyball league. An 8-week administration protocol was assessed on a weekly basis according with the training schedule (Table I). Participants (N.=10) were randomly assigned to either the complexed GBE group (N.=5) or the placebo group (N.=5). Collection of saliva samples and body composition measurements were performed at baseline and at the end of the study.

The acute administration study consisted in 7 days of treatment followed by 7 days of wash out. The 6 elite female volleyball players recruited in this study were involved in a daily standardized training session (135 minutes) composed of specific modules: stretching, volleyball drills, tactics and team play. The treatment (2 capsules of standardized, combined GBE) was provided before each training session. Collection of saliva samples was carried out after each 7-day period (treatment and wash out). In particular, saliva samples were collected before and after the standardized training session of the last day of the corresponding period.

All participants gave written informed consent before enrolment in this study. All procedures received local ethics committee approval, in accordance with the latest version of the Declaration of Helsinki.

Treatment formulations

Virtiva® (Indena SpA, Milan, Italy), composed of standardized GBE and phosphatidyl-

TABLE I.—*Weekly administration protocol of standardized GBE extract complexed with phosphatidylserine (Virtiva®).*

Week Day	Training session/day	Capsules/day
Monday	0	0
Tuesday	2	2
Wednesday	2	2
Thursday	2	2
Friday	2	2
Saturday	1	4
Sunday	Match	4

serine according to the Phytosome® technology,[16] has the following specifications: >5% ginkgo-flavone-glycosides, >0.5% to <2.5% ginkgo-terpenes, >12% phosphatidylserine. Virtiva® was encapsulated in conventional hard-gelatin capsules at the dosage of 240 mg (expressed in weight of the complex). Identical placebo capsules were prepared using inert filling material.

Collection of saliva samples and cortisol concentration measurement

Salivary steroid measurements have the potential to provide a convenient and non-invasive assessment of serum "free" steroid concentrations. Moreover, strong correlations between salivary and total cortisol have been reported.[17]

Saliva samples were always collected at the same time-point and in fasting condition to avoid circadian variation and meal time effects. Participants were asked to rinse their mouth before saliva collection and analysis (Immulite 2000 Immunoassay System, Siemens) as a standard procedure.

Body composition measurement

Age, body composition and body weight were collected, and skin fold fat was measured at seven different body sites (chest, axilla, triceps, subscapular, abdomen, suprailium, thigh). Then the fat body percentage and the fat-free body percentage were calculated using the Jackson and Pollock 7-site skinfold method.[18]

Statistical analysis

Data were analyzed by descriptive statistics. Statistical analysis of cortisol, body weight and composition body parameters were performed by using unpaired two-sample Student's *t*-Test or Mann-Whitney U Test, as appropriate. P-values less than 0.05 were considered significant. Statistical calculations were made using Systat Software (Inc. SigmaPlot).

Results

Chronic administration study

Salivary cortisol level in Virtiva®-treated athletes was significantly lower compared to the placebo-treated group, in both post-treatment time-point ($P<0.05$) and in the difference between pre- and post-treatment time-points, reported in Table II as Δ cortisol ($P<0.001$). Moreover, a significant increase of the difference between pre- and post-treatment free-fat mass value (Δ% free fat mass) was also observed in the Virtiva® group ($P<0.01$) (Table II). Similarly, there was a significantly reduced Δ% fat mass in treated participants (-0.78±0.17) compared with untreated participants (+0.08±0.07; $P<0.01$) (Table II).

Acute administration study

The salivary cortisol levels before (4.29±1.14) and after (4.75±1.33) the last washout training session were similar (Δ washout=0.47±0.66) (Table III). The difference of the same parameters related to the 7-day treatment period (Δ

TABLE II.—*Levels of salivary cortisol, body weight and body composition in the 8-week Virtiva® administration study.*

	Pre-treatment (1)	Post-treatment (2)	Δ (2-1)
Virtiva group (N.=5)			
Cortisol (ng/mL)	20.80±3.15	20.46±2.92 *	-0.38±0.30 ***
Body weight (kg)	68.6±2.56	69.3±2.55	-0.7±0.59
Fat mass (%)	15.16±1.03	14.38±0.96	-0.78±0.17 **
Fat-free mass (%)	84.84±1.03	85.62±0.96	+0.78±0.17 **
Placebo group (N.=5)			
Cortisol (ng/mL)	23.24±1.97	29.58±2.32	+6.34±0.8
Body weight (kg)	73.8±2.74	73.1±2.99	+0.70±0.75
Fat mass (%)	16.4±1.68	16.48±1.68	+0.08±0.07
Fat-free mass (%)	83.6±1.68	83.52±1.68	-0.08±0.07

*P≤0.05; **P≤0.01; ***P≤ 0.001 *vs.* pre-treatment.

TABLE III.—*Concentration of salivary cortisol in the acute Virtiva® administration study.*

Player (N.)	Treatment week		Δ treatment	Washout week		Δ washout
	Pre-training	Post-training		Pre-training	Post-training	
1	8.39	4.12	-4.27	6.68	8.15	1.47
2	6.37	8.12	1.75	4.37	3.02	-1.35
3	5.94	3.94	-2	1.55	2.78	1.23
4	3.3	0.62	-2.68	2.74	1.04	-1.7
5	0.34	2.78	2.44	1.9	4.26	2.36
6	8.25	6.24	-2.01	8.47	9.26	0.79

treatment) was negative (-1.13±1.08). Figure 1 shows the difference of the salivary cortisol level collected before and after the last training session of the treatment period (Δ treatment) and the washout period (Δ washout).

Discussion

Previous studies demonstrated that administration of standardized GBE (EGb761) in young healthy individuals exposed to stress stimuli resulted in a reduction of blood pressure elevation and in an inhibition of salivary cortisol increase.[12] Physiological stress as well as psychological stress might significantly contribute to the level of the "stress hormone" cortisol in athletes. In this study, we investigated the effect of phosphatidylserine-complexed GBE (Virtiva®) treatment on the salivary cortisol concentration and body composition in elite female volleyball players. Although the limited sample size and the overall short follow-up period should be taken into account, we showed that Virtiva® 8-week administration prevented the rise of salivary cortisol level associated with prolonged, intensive, competitive physical activity. In the acute setting, lower cortisol levels were reported after the administration of Virtiva® compared with baseline values, but statistical significance was not reached likely due to the small sample size. Further investigations are needed to clarify the effect of acute administration of standardized, complexed GBE on cortisol level.

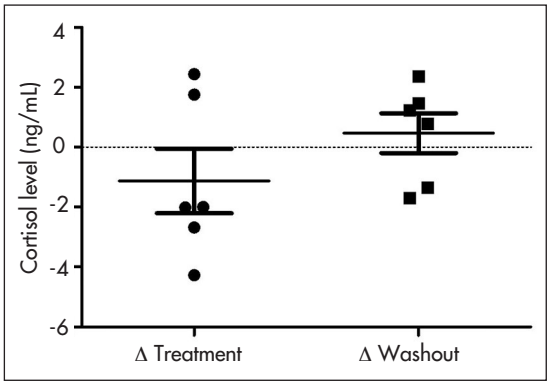

Figure 1.—Difference of the salivary cortisol levels in the acute administration study, as measured before and after the last training session of each 7 days-period (Δ treatment and Δ washout). Values are expressed as mean±SEM.

Prolonged and intensive physical exercise increases lean body mass and decreases body fat. 8-week Virtiva® treatment was associated with a significantly greater increase of fat-free mass and in a significantly greater decrease in percent of fat mass compared with placebo. Therefore, this preliminary evidence may suggest that Virtiva® administration might potentiate the effect of prolonged and intensive physical activity. Currently, it is not known whether this outcome is determined by a direct physiological effect or rather is mediated by an improvement of psychological well-being and cognitive performances of the athletes; a previous study on Virtiva® has demonstrated an important nootropic effect even in single administration in young adults [9] that may, hence, support this second hypothesis. Moreover, we cannot exclude a role played by the sole phosphatidylserine in modulating the athletes' body composition. However, a recent paper seems to exclude this hypothesis.[21]

Cortisol is necessary to respond to physiological and psychological stress. However, chronic elevation of cortisol may have negative effects on different target tissues, such as immune system and skeletal muscle. In particular, glucocorticoids such as cortisol are known

to have catabolic effects on skeletal muscle, either as an endocrine hormone released in response to stress or as a drug given to treat inflammation.[19] In previous studies, herbal supplement administration was tested in order to attenuate the cortisol anticipatory response to impending intense exercise and competition.[20] We suppose that the beneficial effect of 8-week Virtiva® treatment on body composition indexes might result from a reduced catabolic effect due to a decreased stress-induced cortisol release.

Conclusions

Our study suggests that administration of standardized GBE combined with phosphatidylserine (Virtiva®) might exert a beneficial effect on athletes during prolonged intensive training programs by reducing stress induced-cortisol release. However, the mechanisms underlying this effect remains to be further investigated.

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Conflicts of interests.—Stefano Togni and Federico Franceschi are employees of Indena S.p.A. Luca Giacomelli is a consultant of Indena S.p.A. The other Authors declare no conflicts of interest.

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