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EFFECTS OF SUPPLEMENTATION WITH VIRGIN OLIVE OIL ON HORMONAL STATUS IN HALF-MARATHON TRAINED AND UNTRAINED RUNNERS

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ABSTRACT

Several studies have investigated the effects of exercise on hormonal status. Several studies have reported that exercise induce alterations in hormone concentrations. This study focuses on the effects associated to the intake of virgin olive oil, rich in monounsaturated fatty acids (MUFA) on hormonal status in half-marathon athletes. The contents of tocopherols, phenolic compounds, Pigment, flavonoids, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical test and activity of the oil on hydrogen peroxide were determined. The consequence of the consumption of virgin olive oil on hormonal status was studied in healthy male athletes of ages between 19–22 years. The participants were separated into three groups of ten subjects each and reserved under distinct regimes for 10 weeks as follows: Group 1 untrained runners receiving 20 ml of olive oil, Group 2 half-marathon runner performing training routines, 5 days a week while receiving 20 ml of olive oil, Group 3 half-marathon runners performing training routines, 5 days a week unsupplemented with virgin olive oil. Blood samples were taken: one day before endurance training programme, after a 10- week endurance training programme, at the end of the training period, two days before the half-marathon race, and 24 hours after the half-marathon race. Plasma was analyzed for testosterone (T), luteinizing hormone (LH), Follicle Stimulating Hormone (FSH), cortisol (C) and insulin. The results of this study showed that virgin olive oil of Blanquette variety is characterized by high content of tocopherols, phenolic compounds (25.2 ± 0.07 mg/Kg, 485, 46 ± 1.35 mg/Kg), pigments with 79.34.± 0.92 ppm of Total carotenoids, and a high percentage inhibition of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and a percentage of hydrogen peroxide (H_2O_2) inhibition was observed (76.03 ± 0.43% and 86.45±0. 28%, respectively). The consumption of this oil was associated with statistically significant increase of Testosterone in supplemented groups compared with runner of non-supplemented group and sedentary controls. Luteinizing hormone (LH) concentration decreased in runners not supplemented with virgin olive oil compared to group 2 runners and sedentary controls. After a 10-week running training program (before half-marathon race) and immediately after a half-marathon race, cortisol only significantly increased ($p<0.001$) in runners of group 3; it then demonstrated a tendency toward declining 24 hours after a marathon race. This study found that virgin olive oil supplementation can improve hormonal status in half-marathon athletes.

Key words: olive oil, monounsaturated fatty acid, tocopherols, exercise, half-marathon

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INTRODUCTION

Since exercise is a powerful regulator of the release of several hormones, it has been extensively studied as a trigger for hormonal production [1]. Physical stress can have an impact on the hypothalamus-pituitary-gonadal (HPG) axis, based on the nature, severity, and duration of the exercise as well as the person's physical health and psychological characteristics [2]. Some authors have discovered no appreciable changes to the semen parameters in relation to how physical exercise affects an athlete's seminal fluid [3, 4]; others have discovered decreased sperm total count, typical sperm motility, and morphology [5]. Depending on the duration and the intensity of exercise, as well as the person's state of fitness, physical activity can have a variety of impacts on serum testosterone levels [6]. Basal testosterone levels are decreased in intensely trained endurance athletes [6].

An appropriate diet enhances physical exertion, athletic ability, and exercise recovery [7]. Supplements containing omega 3 fatty acids, polyphenols, antioxidants, and vitamins are commonly taken to improve health and sports performance [8]. It is thought that the majority of athletes' nutritional requirements can be met by the Mediterranean Diet [9]. Olive oil, a component of the Mediterranean diet, is showing up in rising number of research to have health benefits, including reducing the risk factors for coronary heart disease, avoiding many cancers, and changing immune and inflammatory reactions [10]. This vegetable oil has a variety of components that may explain some of its general medicinal qualities [10]. In addition to having high amounts of monounsaturated fatty acids, phytochemicals like polyphenolic substances, squalene, and alpha-tocopherol are also abundant in olive oil [10]. Canola and olive oil are the two nutritional oils that are most commonly consumed [11]. Since they are present in large quantities in the above common oils, there is no need to purchase MUFA supplements [12].

The aim of this study was to investigate the effects of consumption of virgin olive oil of Blanquette variety, rich in unsaturated fatty acids and antioxidant micronutrients, on hormonal status in trained and untrained half-marathon runners, before and during a semi-marathon race.

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MATERIALS AND METHODS

Olive oil

The virgin olive oil used in this work was of the Blanquette variety, which accounts for about 20% of the olive orchards in eastern Algeria. This oil was purchased from a traditional oil mill. It is rich in unsaturated fatty acids (oleic and linoleic) [13]. The free acidity, peroxide values and iodine number of this oil are within the limits established by the International Olive Oil Council [13] (Table1).

The total phenolic compounds were determined according to the method recommended by Vasquez [14]. The determination of the flavonoids was carried out according to the method of Branz [15]. The DPPH test was carried out according to the method described by Bektas *et al.* [16]. Hydrogen peroxide scavenging assay was carried out following the procedure of Benkeblia [17] whose principle consists of monitoring the decrease in the concentration of H_2O_2 by spectrophotometry.

Selection of subjects and ethical consideration

Thirty healthy male athletes of ages between 19–22 years voluntarily enrolled in the study. Only healthy male runners without any cardiovascular risk factor were included. All subjects had run a half-marathon through the previous year. Before the start of the research protocol, all the subjects gave their informed consent for contribution in the study. Ethical approval for the study was obtained through the institutions' Human Research Ethics Committee and the study was performed in accordance with the 1964 Declaration of Helsinki. The participants were recruited from a group of runners with varying body mass index (BMI), the baseline physical characteristics of runners are shown in (Table 2) undergoing four weeks running training before completing a half-marathon race. Included in the study were healthy male semi-marathon runners (n=30), divided into three groups of ten subjects each, reserved under distinct regimes for 10 weeks as follows:

- Group 1: untrained runners receiving 20 ml/day of virgin olive oil of Blanquette variety.
- Group 2: semi-marathon runners performing training routines 5 days a week, while receiving 20 ml/day of olive oil of Blanquette variety.
- Group 3: semi-marathon runners performing training routines 5 days a week and unsupplemented with virgin olive oil.

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The virgin olive oil supplement was well tolerated by all participants. No one complained about any negative side effects. In Groups 2 and 3, the same training routines were adopted.

Training protocol

The 10-week running endurance training, for trained groups, comprised continuous aerobic and interval training counting warm-up and cooldown periods with a gradual increase in period and intensity during the course of the 10- week training programme. An individual training programme for each participant was prescribed based on current fitness levels.

Semi-marathon runners' weekly training plan was 4 h 38 min ±1 h 54 min. Weekly exercise in the group of untrained runners was below 2h. Training levels and training duration of untrained runners group corresponds to the general recommendation for weekly exercise. Running exercise duration was different for the different runner groups. Median duration to complete the semi-marathon run was 2 h 11 min 32 sec (±13 min 22 sec). Untrained runners' group was asked to run for 1 hour/week in an athletic stadium, being at their endurance limit at the end of the exercise.

Study protocol

The fasting blood samples were taken according to the protocol described by Mündermann [18]. A sample was taken one day before and after a 10-week endurance training programme (Table 3). In order to collect the blood sample at the end of the training session two days prior to the half-marathon race, the training program was planned for the 10 weeks immediately preceding the marathon race. Prior to the blood test, participants fasted, and the day before the run, they avoided intense physical activity. Additional blood samples were collected directly following the half-marathon run and 24 hours later.

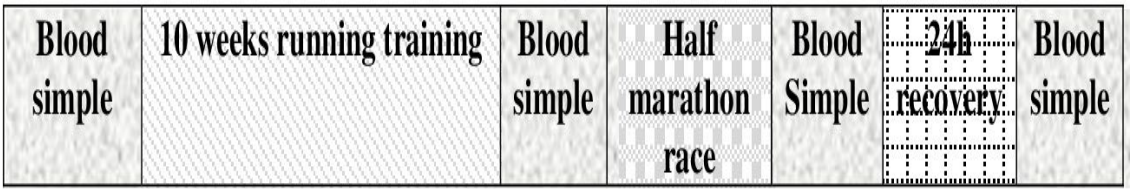

Figure1: The study protocol involved blood samples before and after a 10-week endurance training and a half-marathon race

Hormone Analysis

Whole blood was centrifuged to separate the serum for the hormonal status. Blood samples were collected at the same time of day in order to reduce the effects of

the circadian cycle on hormonal secretion. Serum samples were examined for testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and cortisol. Orion Diagnostica's reagent kits were used for the radioimmunoassay (RIA) analysis of testosterone and cortisol (Turku, Finland). The coefficient of intra-assay variation (CV_{intra}) and of inter-assay variation (CVinter) was 6% and 8%, respectively, for testosterone, and 4% and 8%, respectively, for cortisol. Fluoroimmunometric methods, employing reagent kits from Wallac Oy (Turku, Finland), were used to determine LH and FSH. The CV_{intra} for LH and FSH was < 2%, and CV_{inter} were 4.5 % and 2.9%, respectively. Insulin was analyzed by RIA using an INSULIN-CTMP Biomedicals kit, and gave CVintra and CVinter values of 8% and 11%, respectively.

Statistics

The Statistical Package for the Social Sciences (SPSS) version 20.0 was used to conduct the statistical analysis (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The results were reported as mean ± standard deviation (SD). For group comparison, the Kruskal-Wallis analysis of variance was used, and the Mann-Whitney U test was used for those with $p<0.05$.

RESULTS AND DISCUSSION

Results show that virgin olive oil of the Blanquette variety is rich in total phenolic compounds. For the pigment contents according to Table 3, virgin olive oil of the Blanquette variety, recorded a very high carotenoid content. Results also show that the olive oil studied exerts a high percentage inhibition of the DPPH radical and a percentage of H_2O_2 inhibition exceeding 80%.

Mean circulating levels of T, FSH, LH, Cortisol, and Insulin for the three groups are shown in Table 4. Runners of group 3 had lower serum levels of T compared with runners of group 2 and sedentary controls ($P< 0.001$). For Cortisol, significant increases were observed in runners in the group not supplemented with virgin olive oil. Luteinizing hormone (LH) concentration decreased in runners not supplemented with virgin olive oil compared to group 2 runners and sedentary controls. This change was statistically significant ($p <0.01$). However, there were no significant differences in serum levels of FSH and insulin among the three groups.

Physical activity can have a range of effects on male reproductive function depending upon the intensity and duration of the activity and the fitness of the individual. In this study, results showed that group 3 demonstrated low

testosterone and LH. The concentration of these hormones decrease during intensive exercise [19]. There may be a relation between endurance exercise and reductions in testosterone levels in the blood. Since testosterone has important anabolic functions, changes in reproductive hormone levels may have deleterious skeletal effects comparable to those seen in females with menstrual disruptions [20]. Exercise-induced hormone reaction is dependent on a number of variables, including the subject's training status, mode, duration and method of exercise [21]. The male gonadotrophin reaction to acute exercise is also controversial, short-term exercise increases testosterone levels; however, prolonged exercise, such as military training or marathon racing, decrease these levels [22]. In elite male rowers, basal testosterone was independently associated with weekly exercise intensity. Additionally, it has been proposed that short-term, intense exercise may cause a malfunction of the hypothalamus and pituitary, leading to an altered reaction to exercise [23]. Resistance training caused reductions in total and free testosterone levels similar to the effects of acute resistance training [24]. In this study, the highest levels of testosterone and LH was observed in group 2 which was supplemented with virgin olive oil ($p<0.01$). Ten-week training and virgin olive oil supplementation resulted in increase of testosterone and LH levels. Testicular fatty acids have been shown to have an effect on the histology and physiology functioning of this tissue [8]. Additionally, it has been demonstrated that adding polyunsaturated fatty acids (PUFA) to diets significantly change the composition of the lipids in the testicles, changing how C20–22 fatty acids are metabolized and having a significant effect on the physiology of germinal and steroidogenic cells [25]. Vitamin E deficiency has long been known to cause sterility in animals, and it now appears that this nutrient may be essential for the in vitro growth of Leydig cells [26]. Another research has verified that adding vitamin E to pig Leydig cells in vitro has no impact on the production of basal T [27]. But when coupled with HCG (human chorionic gonadotropin), this vitamin increases the production of testosterone [27]. Vitamin E increases the diameter of the basement membrane, the quantity of spermatogonia and spermatocytes, as well as the diameter and height of the germinal epithelium in rats [28]. Additionally, in the same species, a vitamin E deficit for six months lowers the synthesis of steroid hormones in the adrenal gland and the intracellular transfer of cholesterol [29]. Steroidogenic proteins, which stimulate T biosynthesis, may be produced by vegetable lipids [30]. During Leydig cell steroidogenesis, a combination of mechanisms from cholesterol synthesis, cholesterol esters hydrolysis, and cholesterol conversion to T are joint. Additionally, in Leydig cells, the protein star (steroidogenic acute regulatory protein) supports the passage of free cholesterol from the cytoplasm to the inner mitochondrial membrane, which initiates the steroidogenic process [31]. Olive oil may, therefore, either directly trigger those proteins by boosting their activity or

genetic expression, or indirectly by stimulating the pituitary-testicular axis [32]. The best genetic expression of the enzymes involved in T production, using cholesterol in the testes is activated by luteinizing hormone (LH) [33]. Following two pathways, LH is able to activate Gs protein and induce the transduction of the signal to the nucleus; using phosphorylation by PKA (protein kinase A) and using arachidonic acid [34].

There is controversy regarding the male gonadotrophin reaction to intense exercise [20, 22, 35]. Short-term exercise increases testosterone levels; however, longer-term exercise, such as marathon racing or military training, causes these levels to decrease [22]. This increase in testosterone seems to be simultaneous with any increase in LH, rather than following it, and may, therefore, not be mediated by gonadotrophin secretion [22]. Therefore, the rich composition of virgin olive oil in tocopherols could be the reason of the difference in the androgen hormonal profile observed in the sample. Additionally, it has been noted that some fatty acids and polyphenols obtained from plants prevent testosterone from being converted into dihydrotestosterone by acting as 5 alpha-reductase inhibitors [36]. By activating the hypothalamo-pituitary-testicular system and/or inducing steroidogenic proteins, olive oil may stimulate the production of testosterone. At least in part, Tocopherols, whose mode of action is still unknown, could be attributed for this steroidogenesis [32].

In the present research, 10 weeks of physical training showed a significant increase in resting cortisol compared to groups 2 and sedentary controls. However, in this study, all of subjects' values were within reference limits. While there were no significant differences in cortisol levels between runners in the group supplemented with virgin olive oil and those of sedentary controls, two very important hormonal markers of chronic stress and fatigue are Testosterone (as anabolic hormone) and Cortisol (as catabolic hormone). The relations between these two hormones are thought to reflect the balance between anabolic and catabolic processes. Cortisol, the primary stress hormone was used in many studies to evaluate exercise induced-stress. Exercise intensity and duration have a beneficial impact on the release of the stress hormone cortisol [37]. Cortisol levels at rest typically rise with exercise frequency and intensity. Exercise at a level that exceeds 60% of a person's maximum oxygen uptake (VO2max) is one of the physical stresses that can result in a rise in cortisol secretion [26, 38]. In this study, supplementation with virgin olive oil reduced the cortisol concentration. For the majority of animals, vitamin E is a necessary and essential micronutrient that also has antioxidant qualities. Maintaining the quality of the flesh, immunity, the normal resilience of red blood cells to hemolysis, the preservation of normal capillary

permeability, and cardiac muscle are all essential [39]. In this study, 10-weeks training were not accompanied by elevated insulin in all groups. Other endogenous systems control circulating plasma immunoreactive insulin (IRI) after exercise [40].

CONCLUSION

In conclusion, the findings indicate that virgin olive oil of the Blanquette variety is rich in total phenolic compounds, carotenoid and tocopherol content. Concerning the DPPH technique, this oil is very active in scavenging free radicals. The results are promising and encouraging, reflecting the richness of the virgin olive oil, which can play an important role in the life of athletes, considering the nutritional and therapeutic virtues of these compounds. For better valorization, the effects of virgin olive oil supplementation on hormonal status in half-marathon trained and untrained runners were studied. Results suggest that virgin olive oil supplementation enhances testosterone values and reduces Cortisol levels in half-marathon trained runners. That, in turn, results in protecting or restoring fertility.

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Table 1: Fatty acid composition, free acidity, peroxide values and iodine number of virgin olive oil [13]

Fatty acid type	Fatty acid name	Fatty acid composition (%)
Saturated fatty acids (SFAs)	Myristique C14 :0	0.189± 0.002
	Palmitique C16 :0	16.283±0.001
	Heptadécanoique C17 :0	0.173±0.001
	Stéarique C18 :0	3.732±0.002
	Arachidique C22 :0	0.673±0.001
	Béhénique C22 :0	0.452±0.001
	Lignocérique C24 :0	0.085±0.001
Mono-unsaturated fatty acids (UFAs)	Palmitoléique C16 :1	2.123±0.002
	Heptadécénoique C17 :1	0.189±0002
	Oléique C18 :1	62.087±0.001
	Vaccinique C18 :1	3.081±0.001
	Gadoléique C20 :1	0.231±0.001
Polyunsaturated fatty acid (PUFAs)	Linoleique C18 :2	9.97±0.001
	Linolénique C18 :3	0.732±0.000
Trans fatty acids	Elaidique C18 :1	ND
percentage of the total fatty acids		
- SFAs		21.587±0.001
- UFAs		78.413±0.001
- MUFAs		67.711±0.001
- PUFAs		10.62±0.003
Indices values		
- peroxyde value (meqO_2/Kg)		7.68±1.02
- Free acidity (%)		2.98±0.03
- Iodine number (wijs)		79.23±1.02

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Table 2: Demographic and Training Characteristics of the runners

Variable	Runner (n=30)
Age (years)	20.6 ± 1.2
Heigt (cm)	174 ± 2.3
Weight (Kg)	62.5 ± 3.9
BMI (Kg/cm^2)	21.76 ± 1.7
Mileage (km/wk)	102.3 ± 3.8
Training hours (h/wk)	9.8 ± 1.5
Years training (y)	5.5 ± 1.2
Age began training (y)	16.1 ± 1.1

Table 3: The analytical parameters of olive oil of the Blanquette variety

Parameters	Olive oil
Pigment content (ppm) - Chlorophyll - Total carotenoids	 1.82± 0.5 79.34.± 0.92
Flavonoids content (mg E.Q/Kg)	13,21 ± 0.08
Tocopherol content (mg/Kg)	25.2 ± 0.07
Phenolic compounds (mg /kg)	485,46 ± 1.35
DPPH inhibition (%)	76.03 ± 0.43
H_2O_2 inhibition (%)	86.45±0. 28

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Each value represents the mean ± standard deviation (n = 3)

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Table 4: Circulating and Urinary Hormone Profile in Endurance-Trained Runners, Resistance-Trained Weight Lifters, and Sedentary Controls

Hormonal status	Groups	Baseline	After a 10-week running training programme (before half-marathon race)	Immediately after a half-marathon race	24 h after a marathon race
Testosterone (nmol/l)	G1	$16.3^{a} \pm 0.8$	$16.2^{a} \pm 0.7$	$16.4^{a} \pm 0.5$	$16.2^{a} \pm 0.2$
	G2	$16.4^{a} \pm 0.5$	$20.5^{b} \pm 0.5$	$20.3^{b} \pm 0.7$	$21.8^{b} \pm 0.4$
	G3	$16.6^{a} \pm 0.7$	$12.3^{b} \pm 0.4$	$10.2^{c} \pm 0.3$	$12.7^{d} \pm 0.8$
	P value	> 0.05	< 0.01	< 0.01	< 0.01
luteinizing hormone (LH) (IU/l)	G1	$8.22^{a} \pm 0.2$	$8.20^{a} \pm 0.2$	$8.21^{a} \pm 0.5$	$8.21^{a} \pm 0.3$
	G2	$8.18^{a} \pm 0.5$	$8.25^{b} \pm 0.2$	$8.28^{b} \pm 0.4$	$8.27^{b} \pm 0.2$
	G3	$8.32^{a} \pm 0.4$	$4.26^{b} \pm 0.3$	$4.02^{c} \pm 0.3$	$5.17^{d} \pm 0.2$
	P value	> 0.05	< 0.01	< 0.01	< 0.01
follicle stimulating hormone (FSH) (IU/l)	G1	6.3 ± 0.9^{a}	$6.2^{a} \pm 1.02$	$6.3^{a} \pm 0.8$	$6.5^{a} \pm 0.9$
	G2	$6.7^{a} \pm 1.03$	$6.6^{a} \pm 0.9$	$6.6^{a} \pm 0.8$	$6.8^{a} \pm 0.8$
	G3	$6.9^{a} \pm 1.01$	$6.8^{a} \pm 1.03$	$6.9^{a} \pm 0.9$	$6.7^{a} \pm 1.01$
	P value	> 0.05	>0.05	>0.05	>0.05
Cortisol (nmol/l)	G1	$104.16^{a} \pm 0.43$	$103.24^{a} \pm 0.89$	$106.12^{a} \pm 0.75$	$104.28^{a} \pm 0.48$
	G2	$109.36^{a} \pm 0.25$	$96.7^{b} \pm 0.58$	$97.36^{b} \pm 0.75$	$96.24^{b} \pm 0.67$
	G3	$107.53^{a} \pm 0.57$	$189.36^{b} \pm 0.87$	$299.25^{c} \pm 0.92$	$180.85^{d} \pm 0.97$
	P value	➢ 0.05	< 0.001	< 0.001	< 0.001
Insulin (pmol/l)	G1	$34.72^{a} \pm 0.12$	$33.26^{a} \pm 0.23$	$36.23^{a} \pm 0.17$	$35.12^{a} \pm 0.35$
	G2	$32.89^{a} \pm 0.24$	$34.23^{a} \pm 0.54$	$33.89^{a} \pm 0.65$	$32.65^{a} \pm 0.23$
	G3	$35.26^{a} \pm 0.13$	$34.36^{a} \pm 0.56$	$35.69^{a} \pm 0.26$	$34.97^{a} \pm 0.71$
	P value	> 0.05	> 0.05	> 0.05	> 0.05

Values are mean ± SD (standard deviation); n=3. The means with the same code do not differ, p>0.05). $^{a, b, c;d}$ Significantly difference compared to rest

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