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Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors

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Abstract

Inclusion of biophenols in traditional foods transforms them into functional foods that may help to decrease CVD risk. The aim of the present study was to determine whether the consumption of hydroxytyrosol-enriched sunflower oil (HSO) improves certain CVD biomarker values. A total of twenty-two healthy volunteers participated in a cross-over study involving two 3-week periods, separated by a 2-week washout period, in which volunteers consumed 10–15 g/d of either HSO (45–50 mg/d of hydroxytyrosol) or non-enriched (control) sunflower oil. Total cholesterol, LDL-cholesterol, HDL-cholesterol, arylesterase activity, oxidised LDL and soluble vascular cell adhesion molecule (sVCAM-1) levels were measured in the plasma obtained at the beginning and at the end of each treatment period. The HSO group displayed a significantly higher level ($P<0{\cdot}01$) of arylesterase activity and significantly lower levels of oxidised LDL and sVCAM-1 (both $P<0{\cdot}05$) than the control group. These results suggest that HSO may help prevent CVD.

Key words: Functional foods: Hydroxytyrosol: CVD: LDL: Arylesterase activity: Oxidised LDL: Vascular cell adhesion molecule

Hydroxytyrosol (3,4-dihydroxy ethanol) is a pharmacologically active biophenol present in olive oil (virgin and extra virgin olive oils are its main dietary sources) as a result of the degradation of oleuropein[1]. Scientific evidence suggests that hydroxytyrosol is absorbed even when consumed at moderate doses[2], and its bioavailability is relatively high[3].

Numerous studies on the biological activity of this molecule have demonstrated its great antioxidant capacity, oxidising itself into a catechol quinone[4,5]. Moreover, experimental studies in animals[6] and human subjects[1,7] have demonstrated that hydroxytyrosol improves the lipid profile and antioxidant status, slowing down the development of atherosclerosis. This compound may also reduce the expression of vascular cell adhesion molecule[8] and inhibit platelet aggregation in rats[9] and hypercholesterolaemia in human subjects[10].

Arylesterase activity, a potentially suitable antioxidant biomarker[11,12], has also been tested. Individuals with coronary artery disease[13] and those with familial hypercholesterolaemia display low arylesterase activity levels, therefore, increasing the risk for cardiovascular events[14,15]. Diabetics have also displayed reduced arylesterase activity, although this finding may be attributable to a decrease in paraoxonase 1 (PON-1) expression in these individuals[16].

On the other hand, hydroxytyrosol would counterpart the potential pro-oxidant effect of sunflower oil[17] and maintain the hypocholesterolaemic properties of sunflower oil, rich in linoleic acid[18].

These premises induced us to study the use of hydroxytyrosol as a potential functional ingredient. Added to a suitable matrix such as culinary oil, this biophenol may display beneficial properties. The SOS Group has developed hydroxytyrosol-enriched sunflower oil (HSO, Oleoactive®), which may be considered a potential functional food. The aim of the present study was to determine the effects of Oleoactive® consumption on the lipoprotein profile, PON-1 arylesterase activity, oxidised LDL levels and soluble vascular cell adhesion molecule (sVCAM-1) levels in a sample of healthy volunteers.

Abbreviations: PON-1, paraoxonase 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

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Subjects and methods

HSO and control sunflower oil, both from the same manufacturer (Koipesol; SOS Group SA, Madrid, Spain), were studied. After signing an informed consent form, as stipulated by the ethical guidelines of the Helsinki Declaration, twenty-four healthy volunteers (seventeen women and seven men) between the ages of 20 and 45 years enrolled in the study. The study design was accepted by the Ethical Committee of Puerta de Hierro University Hospital, Madrid, Spain.

Participants had to fulfil the following eligibility criteria: age between 20 and 45 years and BMI between 18 and 33 kg/m^2. Exclusion criteria included those with familial monogenic hypercholesterolaemia, diabetes type 1 and 2, hypothyroidism; those taking any hypolipaemiant, hypertensive or anti-inflammatory drugs and those with habitual high consumption of alcohol. Study participants were randomly assigned to one of two groups to follow a single-blinded, cross-over, placebo-controlled study, consisting of two 3-week experimental periods. Participants followed their normal dietary habits during the 2-week washout interval that separated the two trial periods. During the experimental period, volunteers, according to their body weight, consumed 10–15 g/d of HSO (45–50 mg/d of hydroxytyrosol). During the control period, study participants consumed 10–15 g/d of control sunflower oil. Two of the participants did not complete both experimental periods; thus, data analysis was performed in twenty-two volunteers (sixteen women and six men). All participants developed moderate physical activity and did not change their habitual physical activity throughout the study.

Four blood samples, one at the beginning and another at the end of each treatment period, were obtained from all volunteers. Serum or plasma was obtained after centrifugation at 3000 rpm at 4°C for 20 min. Aliquots of serum or plasma were kept at −80°C until analysis.

Diet assessment

Food consumption and diet quality were evaluated by means of two 24 h dietary records and dietary intake frequency, one at each dietary period. Participants recorded the amount and kinds of food consumed every day to avoid any possible doubt regarding their diets. With the consent of the study participants, the surveys were checked by a trained dietitian. Food composition tables were used to calculate the volunteers' dietary energy and nutrient intakes[19]. Special emphasis was given to compliance and management of intake with regard to frequency, date and number of oil doses consumed and to constancy in dietary habits throughout both experimental periods. Participants preferentially used HSO and the control oil as a supplement for dressing.

Anthropometric measurements

Trained staff measured weight, height and BMI of the participants at the start and at week 3 of both dietary periods.

Determination of serum cholesterol and lipoprotein cholesterol profiles

Total cholesterol and HDL-cholesterol were measured with their respective enzymatic kits from Roche Diagnostics (Hitachi, Tokyo, Japan), using a Hitachi 917 autoanalyser. LDL-cholesterol concentrations were calculated using the Friedwald *et al.* equation[20].

Determination of arylesterase activity

Arylesterase activity was measured using simulated body fluid, a mimetic buffer of human plasma, at 37°C by means of the Nus method[11]. One unit of arylesterase was defined as the mmol of phenol formed from phenyl acetate per min monitored using a thermostated T80 + spectrophotometer from PG Instruments® (PG Instruments Limited, Wibtoft, Leics, UK).

Determination of oxidised LDL

Oxidised LDL in the samples was determined using an ELISA kit from Mercodia Laboratories (Uppsala, Sweden). The colorimetric endpoint was measured at 450 nm using a spectrophotometer (model ELx808 BioTek®; BioTek Instruments, Winoosky, VT, USA).

Determination of soluble vascular cell adhesion molecule 1

sVCAM-1 concentrations of patients and control subjects were measured by ELISA using reagent kits from Diaclone Research (Besançon, France).

Statistical analysis

Data are expressed as means and standard deviations. Results were evaluated using the SPSS 15.0 Statistical Analysis software package (SPSS, Inc., Chicago, IL, USA). A repeated-measures ANOVA using treatment as a factor was performed, followed by Student's *t post hoc* test. Data were accepted as significant at $P \leq 0{\cdot}05$.

Results and discussion

This is the first time that the effect of HSO consumption on the arylesterase activity of PON-1 has been studied and defined. Moreover, to the best of our knowledge, no studies have tested the effect of HSO on CVD markers.

Diet and nutrient consumption

Table 1 shows the characteristics of the diet consumed by the study participants. The diet presents an energy profile similar to that of the average Spanish diet, including high-lipid, high-MUFA and low-carbohydrate contributions[21]. The macronutrient content of the diet or antioxidant vitamin composition did not significantly differ between the experimental periods.

Table 1. Energy, selected nutrient and fibre intakes*
(Mean values and standard deviations, *n* 22)

	Hydroxytyrosol-enriched sunflower oil		Control sunflower oil		
	Mean	SD	Mean	SD	*P*
Energy (kJ)	11 165·2	3566·7	11 204·3	3579·2	NS
Carbohydrates (g/d)	288·84	97·02	296·84	97·36	NS
Protein (g/d)	109·57	33·72	115·37	33·84	NS
Lipids (g/d)	115·50	44·23	116·92	44·38	NS
MUFA (g/d)	51·54	22·52	54·43	23·78	NS
PUFA (g/d)	14·87	5·91	14·66	5·82	NS
SFA (g/d)	37·97	15·42	37·15	15·09	NS
Alcohol (g/d)	5·06	3·88	4·85	3·72	NS
Fibre (g/d)	22·04	7·73	23·80	8·35	NS
Carbohydrates (% En)	43·09	4·95	41·57	4·78	NS
Protein (% En)	16·78	3·09	17·23	3·17	NS
Lipids (% En)	38·64	5·17	39·30	5·26	NS
MUFA (% En)	17·20	3·69	18·29	3·92	NS
PUFA (% En)	5·01	0·98	4·93	0·96	NS
SFA (% En)	12·63	2·29	12·49	2·26	NS
Alcohol (% En)	1·37	1·02	1·27	0·94	NS
Vitamin E (mg/d)	10·27	4·12	10·79	4·18	NS
Vitamin C (mg/d)	140·54	53·87	142·27	54·01	NS
Vitamin A† (mg/d)	729·33	279·30	738·33	280·24	NS

% En, percentage of energy.
* Repeated-measures ANOVA using treatment as a factor.
† As retinol equivalents.

Body weight and BMI were not affected by HSO consumption (data not shown). Polyphenols may stimulate energy expenditure by increasing thermogenesis. However, 3 weeks may be considered too a short period of time to significantly affect anthropometrical characteristics, even though taking into account that dietary habits, energy consumption and wasting were not modified through the study.

Cholesterol and lipoproteins

HSO was unable to modify serum cholesterol and lipoprotein concentrations (Table 2). These results contrast with those of the Eurolive study[7] in which olive oils with different levels of polyphenols were studied. In that study, olive oil rich in polyphenols reduced LDL-cholesterol and TAG levels and increased HDL-cholesterol levels. Taking into account that the amount of hydroxytyrosol tested in the present study was higher than that used in the Eurolive study[7,22], it can be suggested that oil type can be considered an important determinant when selecting a matrix for this functional ingredient.

Oxidised LDL

Oxidised LDL values ($P=0·05$) and the oxidised LDL:total LDL-cholesterol ratio ($P=0·008$) were significantly lower in individuals who consumed HSO than in those consuming the control oil (Table 2). The antioxidant properties of hydroxytyrosol,

Table 2. Results of different determinations*
(Mean values and standard deviations, *n* 22)

	Hydroxytyrosol-enriched sunflower oil				Control sunflower oil				
	Baseline		3 weeks		Baseline		3 weeks		
Type of oil	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
AE activity (U/l)†	235·18	162·98	448·86	204·12	272·41	169·61	310·32	90·82	0·009
TAG (mmol/l)	1·25	1·93	0·86	0·34	0·93	0·35	0·85	0·41	0·391
HDL-cholesterol (mmol/l)	1·51	0·31	1·40	0·25	1·45	0·26	1·39	0·28	0·365
LDL-cholesterol (mmol/l)	2·74	0·68	2·88	0·75	2·91	0·71	2·93	0·73	0·489
Oxidised LDL (U/l)	79·79	63·73	64·14	57·61	72·72	59·23	86·45	60·08	0·05
Oxidised LDL:total LDL-cholesterol (U/mmol)	33·7	33·4	22·4	15·5	27·5	23·2	32·5	25·1	0·008
VLDL-cholesterol (mmol/l)	0·70	1·10	0·39	0·15	0·42	0·16	0·39	0·18	0·205
Total cholesterol (mmol/l)	4·92	0·96	4·66	0·77	4·78	0·75	4·70	0·90	0·161
sVCAM-1 (ng/ml)	679·26	134·92	645·42	113·54	592·40	132·54	634·67	149·93	0·021
AE:HDL-cholesterol (U/mmol)	155·50	107·37	320·38	149·51	187·28	93·64	224·01	106·11	0·009

sVCAM-1, soluble vascular cell adhesion molecule; AE arylesterase.
* To transform TAG and cholesterol in mg/dl, multiply present data by 89 and 38·7, respectively. Repeated-measures ANOVA using treatment as a factor.
† One unit of AE was defined as the mmol of phenol formed from phenyl acetate per min at 37°C.

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combined with increased PON-1 activity, significantly reduced the serum levels of these oxidised lipoproteins, which are very important in the development of atherosclerosis. Previous studies in humans have revealed that consumption of biophenols from olive oil reduces oxidised LDL levels[1,7].

Arylesterase activity differences

Arylesterase activity was significantly ($P=0{\cdot}009$) greater during the HSO trial period than during the control period (Table 2). The present results coincided with those of *in vitro*[4] and experimental animal[6] studies that investigated the antioxidant activity of hydroxytyrosol. The free radical-scavenging capacity of hydroxytyrosol counteracts lipid peroxidation and helps to improve antioxidant status[4,5]. PON-1, defined as a suicide enzyme[23], assures the continued antioxidant activity of other enzymes. Consumption of HSO may thus help improve antioxidant status by increasing arylesterase. The arylesterase activity:HDL-cholesterol ratio increased significantly ($P=0{\cdot}009$) during the HSO trial period (Table 2). These data suggest that antioxidant capacity of HDL[12,24] increases following the consumption of the HSO diet.

Soluble vascular cell adhesion molecule 1 levels

sVCAM-1 levels were significantly lower ($P=0{\cdot}021$) during the HSO trial period than during the control period (Table 2). Adhesion molecules have been implicated in leucocyte–endothelium interactions, which lead to the formation of atherosclerotic plaques[24]. Reduced expression of vascular cell adhesion molecule suggests low CVD risk[25], as this molecule plays an important role in the development of atherosclerosis and in inflammatory processes. As oxidised LDL is known to increase vascular cell adhesion molecule expression and levels[8,24], the drop in oxidised LDL levels in the study participants consuming HSO seems to be directly related to the decrease in their sVCAM values.

Conclusions

Although HSO was unable to reduce LDL-cholesterol or increase HDL-cholesterol, it acts as a functional food by increasing arylesterase activity and reducing oxidised LDL and sVCAM-1 levels. This oil can therefore be used as a dietary complement to reduce CVD risk. Further studies are needed to understand the mechanisms by which hydroxytyrosol affects arylesterase activity and influences the arylesterase activity:HDL-cholesterol ratio.

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