Grape extract and α-Tocopherol effect in cardiovascular disease model of Apo E -/- Mice[1]

Efeito do extrato de uva e α-Tocoferol em camundongos Apo E -/-, modelo de doença cardiovascular

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ABSTRACT
Purpose: To verify the effect of consumption of grape extract isolated or combined with α-tocopherol supplementation on atherosclerosis model with Apo E -/- mice. **Methods**: After six weeks of atherogenic diet, Apo E -/- mice were divided into the following groups: Control, Grape, Tocopherol and Grape plus Tocopherol. The treatment progressed for 11 weeks when animals were submitted to euthanasia. **Results**: All the treatments presented hypocholesterolemic effect with reduction of serum and liver cholesterol levels. This effect was parallel to an increase in the fecal excretion of cholesterol. There was also a higher fecal excretion of saturated fatty acids in groups receiving grape extract or α-tocopherol. All the groups treated presented a tendency to show higher levels of vitamin E. The fatty acid profile showed a tendency for monounsaturated fatty acid preservation after grape extract and α-tocopherol consumption. Morphological analysis revealed a lower degree of evolution of the atherosclerotic plaque of the animals that were fed α-tocopherol combined with grape extract, even when no difference was found in the size of the largest lesion. **Conclusion**: A synergistic effect between the polyphenols and α-tocopherol was observed, resulting in diminished evolution of atherosclerosis and a greater beneficial effect on atherosclerosis than the isolated consumption of antioxidants.
Key words: Cholesterol, LDL. Vitis. alpha-Tocopherol. Diet, Atherogenic. Mice.

RESUMO
Objetivo: Verificar o efeito do consumo de extrato de uva isolada ou combinada com a suplementação de α-tocoferol em modelo de aterosclerose, utilizando camundongos Apo E -/-. **Métodos**: Os camundongos Apo E -/- foram tratados com dieta aterogênica por seis semanas e foram divididos em quatro grupos: Controle, Uva, Tocoferol e Uva e Tocoferol. Após 11 semanas de tratamento os animais foram submetidos à eutanasia. **Resultados**: Todos os tratamentos apresentaram efeito hipocolesterolêmico, com redução de colesterol plasmático e hepático. Este efeito foi acompanhado de um aumento na excreção fecal de colesterol. Houve também uma maior excreção fecal de ácidos graxos saturados nos grupos que receberam extrato de uva ou de α-tocoferol. Todos os grupos apresentaram uma tendência a apresentar níveis mais elevados de vitamina E. O perfil de ácidos graxos mostrou uma tendência para a preservação de ácidos graxos monoinsaturados, após consumo de extrato de uva e α-tocoferol. A análise morfológica revelou um menor grau de evolução da placa aterosclerótica dos animais que foram alimentados com α-tocoferol combinado com extrato de uva, mesmo quando não houve diferença no tamanho da lesão. **Conclusão**: Foi observado um efeito sinergístico entre os polifenóis e α-tocoferol, resultando na redução na evolução da aterosclerose e um maior de efeito benéfico na aterosclerose do que o consumo isolado de antioxidantes sobre a aterosclerose do que o consumo isolado de antioxidantes.
Descritores: Colesterol LDL. Vitis. alfa-Tocoferol. Dieta Aterogênica. Camundongos.

Introduction

The main causes of death in the western societies are cardiovascular diseases, whose major manifestations are: heart attacks, embolisms, and cerebral vascular accidents (CVA)[1]. Atherosclerosis is the main alteration involved in the process of cardiovascular diseases. It is characterized by the formation of atheroma plaques from the accumulation of lipids inside the macrophages, which is subsequently deposited in the vascular intima triggering an inflammatory response. This process seems to be directly linked with oxidative modification of the low-density lipoprotein-LDL (Low Density Lipoprotein) in the circulation[2].

Therefore, oxidative modifications within the arterial wall may initiate and/or contribute to atherogenesis. The shift in the balance between oxidants and antioxidants in favor of the former would be one of the factors contributing to inflammatory responses and to a vicious cycle of lipid peroxidation within the lipoproteins and cellular recruitment for uptake of modified lipoproteins from circulation[3].

Many factors could contribute to compromise endogenous antioxidant defense system like high exposure to pathological agents of different origins or even a deficient diet[4]. In the context of the oxidative modification hypothesis, antioxidant protection of LDL in the extracellular space deserves focus, as oxidized LDL has many potential proatherogenic activities. Thus, the use of antioxidants through the diet could be a useful therapeutic measure applicable against LDL oxidative modification.

Compounds able to prevent the consequences of radicals involved in lipid peroxidation through its inactivation could contribute to the maintenance of health or to reduce progression of atherosclerotic lesions. Red wine, white wine and grape juice all have high antioxidant potential to protect cellular structures against peroxidation reactions owing to their rich phenolic contents[5]. Grape juice is a rich source of antioxidant compounds as the flavonoids catechin, epicatechin, quercetin, and anthocyanins[6]. In vitro studies showed that grape juice has sufficient antioxidant activity to inhibit LDL oxidation[7]. At the same time high-dose α-tocopherol supplementation in humans decreases the susceptibility of LDL oxidation. Jialal *et al*.[8] compared the effect of α-tocopherol in doses of 60, 200, 400, 800, and 1200 IU/d, and found that the minimum dose of α-tocopherol needed to significantly decrease the susceptibility of LDL to oxidation is 400 IU/d.

To better understand the action of dietary antioxidants on atherogenesis, this work was carried out to evaluate the influence of diet supplementation with grape juice extracted from Niagara grape (*Vitis vinifera L.*) and α-tocopherol on the development of atherosclerosis in Apo E-/- mice and the likely synergistic effect of the simultaneous use of antioxidants.

Methods

Animals and diets

A total of 49 Apo E gene deficient 57BL/6 male and female mice of same age and similar weights were selected. The 5-week old animals were fed atherogenic diet for six weeks. After this period, the animals were separated into 4 groups with different treatments. The experimental treatments consisted in: a) Control, fed atherogenic diet and water; b) Grape, fed atherogenic diet and Niagara grape extract; c) Tocopherol, fed atherogenic diet supplemented with 400mg of α-tocopherol acetate/Kg of diet and water; and d) Grape /Tocopherol, fed atherogenic diet supplemented with 400mg of α-tocopherol acetate /Kg of diet and Niagara grape extract diet. The diet, grape extract and water were given *ad libitum* and food intake was followed up weekly. The treatment lasted 11 weeks when the animals were euthanized.

The experimental diets were prepared according to A.O.A.C. protocol 1989[9]. The aqueous grape extract was prepared using 1.5 kg of Niagara grape (*Vitis vinifera L.)* produced in northwestern São Paulo, Brazil, and purchased at a market in Viçosa-MG, Brazil. The grapes were processed with seeds in a domestic blender with a total of 20 mL of distilled water and filtrated in a cotton cloth. The extract was stored at -25ºC, protected from light and oxygen. The dilution of the concentrated extract was made in the proportion 1:5 (extract/distilled water) for animal consumption.

Experimental protocols used on this research were developed following Animal Experimentation Brazilian College rules and management recommendations.

Cholesterol and triacylglycerol dosage

Total cholesterol and triacylglycerol dosage in the serum, liver, and feces was performed by means of enzymatic kits (donated by KATAL and Bioclin Laboratories). Cholesterol and triacylglycerol were extracted from liver and feces according to Folch *et al.*[10]

Vitamin E dosage

Serum and liver vitamin E levels were analyzed by High-Performance Liquid Chromatography (HPLC), after vitamin extraction according to the method proposed by Ueda and Igarashi[11].

The equipment consisted of a visible UV diode array detector (Shimadzu), Lichrospher column, RP-18 (4.0 x 250 mm, 5 μm). A mixture of acetonitrila, methanol and hexane was used as mobile phase in the ratio 3:95:2. Running flow was 1 mL/minute and running time was 10 minutes. The wavelength used in the detector corresponded to a maximum absorbance of α-tocopherol, 295 nm. The vitamin standard applied was α-tocopherol (Sigma-Aldrich®, EUA), dissolved in ethanol at 96%, resulting in a stock solution of 100 μg/mL.

Soluble polyphenol dosage

Polyphenol determination was carried out in the grape extract, serum, liver and feces according to the method of Singleton and Rossi[12], using gallic acid as standard.

Determination of the anthocyanins in the grape extract

Anthocyanin content was obtained based on the technique proposed by Lees and Francis[13].

Malondialdehyde (MDA) dosage

Malondialdehyde (MDA) levels were determined in the liver according to the method developed by Gutteridge and Halliwell[14].

Lipid hydroperoxide dosage

Lipid peroxidation sub-products capable of oxidizing ferrous ions (Fe^{3+}) into ferric (Fe^{2+}) were determined in the serum and liver, according to the method proposed by Nourooz-Zadeh *et al.*[15]

Fatty acid profile analysis

After lipid extraction from the liver and fecal tissues, saponification and esterification of the lipid extracts was carried out for gas chromatography (GC) analysis. The methodology applied for saponification and esterification of the lipid extract was proposed by Hartman and Lago[16]. For the chromatographic running, an aliquot of 1μL of the lipid extract solution was injected in hexane (50mg/mL) in CG-17^{A} Shimadzu/Class chromatograph, equipped with SP-2560 (biscianopro2pil polysiloxane) used silica chromatographic column of 100m and 0.25mm diameter. The carrier gas (mobile phase) was nitrogen. Running started with the column temperature at 100ºC, increasing 10ºC/min until reaching 180ºC. After this period, the column temperature increased 1ºC/min until reaching 240ºC, remaining at this temperature for 10 min. The injector and detector temperatures were 250ºC and 270ºC, respectively. Fatty acid identification considered the retention times of a standard fatty acid methyl ester (Supelco™ 37 Component FAME Mix).

Morphological and morphometrical analyses

Morphological analysis of aorta artery was carried out according to classification proposed by Stary *et al.*[17] The presence of specific histopathologic characteristics of six different degrees of atherosclerotic lesion was considered.

The morphometrical analysis was carried out using the area average of the three major lesions of each animal. The optical microscope Olympus Provis U-MCB, coupled to a Spot Insight Color digital camera and computer, was used to capture the images. The images were measured by means of the software Image-Pro Plus 4.5.

Ethics committee

This project was approved by the Ethics Committee of the Department of Veterinary Medicine of the Federal University of Viçosa, processed 13/2008, and the experiment was carried out according to the Ethical Principles in Animal Experimentation, adopted by the Brazilian College of Animal Experimentation (COBEA).

Statistical analysis

The ANOVA analysis of variance and Tukey test was used for comparison of symmetric data. The non-parametric test of Kruskal – Wallis complemented by the Dunn´s test of multiple comparisons was used for asymmetric data. For histological analysis, the statistical Qui-square test was applied. A significant level (p) below 5% was accepted, conferring the study a reliability of 95%. For data analysis, the software Sigma Stat 2.03 and the software EpiInfo 6 was used.

Results

Animals and diets

The results did not show treatment influences on weighted gain, liver relative weight, and food consumption. The levels of

total polyphenols in the pure extract was 331.00 ± 51.00 mg/100g and anthocyanins 248.75 ± 132.45 mg/100g. Animals consumed an average of 9.7 mL daily, with a consumption of 32.11mg of polyphenols and 24.13 mg of anthocyanins. Alpha-tocopherol consumption was estimated to be ten times higher in those groups receiving this vitamin: 0.018, 0.175, 0.019 and 0.179 mg/week/animal in the control, tocopherol, grape, and tocopherol plus grape, respectively.

Cholesterol and triacylglycerol levels

The antioxidants supplementation was effective in influencing total serum cholesterol levels, specially the association of both antioxidants. Treated groups presented 15.71%, 35.8% and 43.5% smaller cholesterol levels in comparison with control group in animals given grape extract, α-tocopherol and both the antioxidants respectively. The smallest content of cholesterol in the liver was found in the group consuming grape extract in association with α-tocopherol, followed by animals fed only α-tocopherol and grape extract. The vitamin supplementation resulted in the highest level of cholesterol in feces samples, and the consumption of grape extract alone also favored presence of higher levels of cholesterol in feces in comparison with control animals (Table 1), suggesting that these treatments might have been effective in exerting hipocholesterolemic effect through fecal excretion mechanism.

The treatments did not influence the serum and liver triacylglycerol levels. However, grape extract intake led to a significant increase in total triacylglycerol fecal excretion compared with control (Table 1).

TABLE 1 - Apo E-/- mice experimental groups' serum, liver and fecal total cholesterol and triacylglycerol levels.

Lipid	Control	Tocopherol	Grape	Grape and Tocopherol
Total Cholesterol	***n= 12***	***n= 9***	***n= 12***	***n= 12***
Serum(mg/dL)	1024.71 ± 83,21	657.36 ± 69.65*	863.74 ± 132.47*	578.56 ± 196.27*†
Liver (mg/g)	25.78 ± 2.61	13.20 ± 3.13*	14.23 ± 3.32*	8.39 ± 2.01*†
Fecal(mg/g)*	14.77 ± 2.52	24.97 ± 0.54*†	22.21 ± 3.63*‡	18.27 ± 1.91*†‡
Triacilglycerol	***n= 6***	***n= 6***	***n= 6***	***n= 6***
Serum(mg/dL)	204.07 ± 90.00	132.21 ± 30.01	146.07 ± 62.77	127.43 ± 45.17
Liver (mg/g)	85.78 ± 24.58	87.41 ± 12.94	63.34 ± 14.67	83.68 ± 24.12
Fecal(mg/g)	2.04 ± 1.07	-	17.21 ± 3.54*	17.91 ± 1.20*

Data are given as means ±SEM * p<0.05 compared with control group. †p<0.05 compared with grape group. ‡p<0.05 compared with tocopherol goup (ANOVA analysis of variance).

Serum and liver vitamin E levels and soluble polyphenol

The use of antioxidants as part of the diet tended to enhance vitamin E serum levels around 2.2, 2.8 and 3.7 times respectively in animals receiving tocopherol, grape extract and tocopherol + grape compared to control group, although no statistical significance was found. While groups receiving grape extract, either isolated or in association with -tocopherol, presented higher vitamin E serum levels, their hepatic content of this vitamin was smaller in relation to control group (Table 2).

The grape extract treatment did not alter the serum and liver levels of polyphenols; however, the groups receiving grape extract presented fecal content of polyphenols smaller than control and tocopherol group (Table 2).

TABLE 2 - Apo E-/- mice experimental groups' serum, liver and fecal vitamin E and soluble polyphenol levels.

Parametrer	Control	Tocopherol	Grape	Grape and Tocopherol
Vitamin E	***n= 6***	***n= 5***	***n= 5***	***N= 7***
Serum (mg/dL) *Stample pool*	2.24	4.95	6.45	8.25
Liver (µg/g)	16.23 ± 1.96	19.79 ± 6.89	6.93 ± 1.70*	8.90 ± 2.27*‡
Soluble polyphenal	***n= 10***	***n= 9***	***n= 10***	***n= 10***
Serum(mg/dL)	4.33 ± 0.81	4.81 ± 0.27	4.15 ± 0.60	4.28 ± 1.47
Liver (mg/g)	6.82 ± 2.66	7.44 ± 3.44	4.29 ± 2.01	4.69 ± 1.84
Fecal(mg/g)	1.68 ± 0.27	1.94 ± 0.07	0.58 ± 0.13*‡	0.70 ± 0.17*‡

Data are given as means ±SEM. * p<0.05 compared with control group. †p< 0.05 compared with grape group. ‡p<0.05 compared with tocopherol goup (ANOVA analysis of variance).

Malondialdehyde and lipid hydroperoxide

The treatments did not influence the liver levels of MDA and the lipid hydroperoxide levels. However, these levels tended to reduce with consumption of both antioxidants (Data not shown).

Liver and fecal fatty acid profile

Interventions with antioxidants had different impact on the fatty acid profile of the liver: supplementation with α-tocopherol preserved oleic fatty acid (C18:1) as compared with grape extract consumption, while the group fed grape extract presented a higher DHA (C22:6) fatty acid percentage (Table 3).

TABLE 3 - Apo E-/- mice experimental groups' liver fatty acid profile.

Fatty acids (%)	Control (n=12)	Tocopherol (n=9)	Grape (n=14)	Grape and Tocopherol (n=13)
Saturated	39.06 ± 21.15	32.62 ± 6.19	24.10 ± 18.72	27.77 ± 17.96
C16:0	36.19 ± 7.72	29.50 ± 5.69	31.87 ± 9.08	31.98 ± 11.09
C18.0	4.94 ± 2.30	4.37 ± 1.63	6.98 ± 3.62	7.45 ± 4.72
Monounsaturated	48.06 ± 5.03	49.13 ± 13.08	36.39 ± 13.66	39.75 ± 9.54
C16:1	48.06 ± 5.03	2.93 ± 1.08	2.46 ± 1.63	2.44 ± 0.99
C18:1	2.51 ± 0.83	47.50 ± 14.13†	32.57 ± 14.30‡	36.97 ± 8.25
Polyunsaturated	43.58 ± 7.77	24.53 ± 11.91	34.35 ± 14.64	31.08 ± 8.51
C18:2 ω6	26.49 ± 7.02	20.02 ± 10.06	12.53 ± 2.12	13.19 ± 2.36
C18:3 ω3	14.57 ± 2.72	ND	0.84 ± 0.37	ND
C20:4 ω6	1.35 ± 0.08	5.78 ± 1.38	11.88 ± 6.16	10.04 ± 4.38
C22:6 ω3	8.20 ± 2.50	4.70 ± 1.94	10.64 ± 6.17	7.48 ± 1.97

Data are given as means ±SEM. * $p<0.05$ compared with control group. †$p<0.05$ compared with grape group. ‡$p<0.05$ compared with tocopherol goup (ANOVA analysis of variance). ND= undetermined value.

Fecal fatty acid profile was reduced in DHA when the animals were fed α-tocopherol, suggesting that this vitamin may contribute to DHA absorption. The antioxidant treatments provided a slight increase in the fecal excretion of saturated fatty acids and smaller excretion of mono and polyunsaturated fatty acids (Table 4).

TABLE 4 - Apo E-/- mice experimental groups' fecal fatty acid profile.

Fatty acids (%)	Control (n=6)	Tocopherol (n=6)	Grape (n=6)	Grape and Tocopherol (n=6)
Saturaded	24.61 ± 18.41	74.41 ± 6.35	37.23 ± 18.53	41.03 ± 37.59
C16:0	17.63 ± 11.13	22.92 ± 1.88	24.89 ± 0.87	22.48 ± 3.43
C18:0	22.03 ± 0.17	46.48 ± 5.06*	37.13 ± 1.95*	30.27 ± 2.08
Monounsaturated	32.00 ± 31.64	20.36 ± 4.70	22.36 ± 24.36	20.43 ± 17.70
C18:1	23.30 ± 19.68	17.84 ± 19.68	15.53 ± 5.84	21.91 ± 7.17
Polyunsaturated	29.47 ± 14.42	23.23 ± 32.18	19.80 ± 2.27	14.37 ± 5.06
C18:2 ω6	8.04 ± 2.51	4.08 ± 0.71	5.22 ± 1.64	5.22 ± 2.92
C22:6 ω3	19.71 ± 4.69	3.71 ± 3.80*	14.58 ± 3.89	9.75 ± 6.48

Data are given as means ±SEM. * $p<0.05$ compared with control group. †$p<0.05$ compared with grape group. ‡$p<0.05$ compared with tocopherol goup (ANOVA analysis of variance).

Morphological analysis of aorta

In the morphological analysis of the sections, no difference was observed in relation to size of the atherosclerotic lesion area, but about the degree of atherosclerotic lesion development. The group receiving the grape extract combined with α-tocopherol didn't present any lesion at advanced stage. Animals receiving just α-tocopherol supplementation showed a better profile of lesion classification in comparison to control and grape extract group (Table 5).

TABLE 5 - Morphological classification of specific histopathological characteristics of six different degrees of atherosclerotic lesion.

Group	Proportion of lesion classified at each stage* development		
	Initial	Intermediate	Advanced
Control (n=12)	0%	50%	50%
Tocopherol (n=8)	37,5%	50%	12,5%
Grape extrac (n=15)	13,3%	53,3%	33,4%
Grape extract and tocopherol (n=15) †	30%	70%	0%

*According to classification proposed by Stary *et al.*[17] †$p<0.05$ compared with control group according to chi-square test.

Discussion

Evidences of polyphenols benefits are usually derived from *in vitro* or animals experiments which usually applies higher doses than those usually consumed by humans[18]. Our treated mice consumed a dose of approximately 1600mg of polyphenols/kg of body weight and 1200 mg of anthocyanins/kg of body weight, a very high quantity if we consider men's average intake of flavonoids assessed by Hertog *et al.*[19] around 25mg/day, which would represents an average dose of 0.33 mg/kg body weight for an average man of 75 kg. At this relative low level of intake, they found that flavonoid intake was significantly inversely associated with mortality from coronary heart disease in humans.

Administration of grape extract and α-tocopherol reduced serum and hepatic cholesterol levels, although this effect has been potentiated when these antioxidants were given together. This hypocholesterolemic effect of the polyphenols was also observed by Yang and Koo[20]. In their study they used polyphenols from green tea and observed a reduction in the levels of serum and liver cholesterol in hypercholesterolemic mice. The same effect was not observed after vitamin E intake in a study developed by Peluzio *et al.*[21],

Cyrus *et al.*[22] and Koga *et al.*[23]. These authors did not observed any reduction in the levels of cholesterol in Apo E-/- rats, LDL -/- rats and rabbits, respectively, treated with different doses of vitamin E.

The hypocholesterolemic effect of these antioxidants may be attributed to the action of these substances on cellular metabolism. Polyphenols found in non-alcoholic wine decrease Apo B lipoprotein synthesis by liver cells, thus decreasing the production of lipid-transporting lipoproteins in the plasma. Both, polyphenols[24] and vitamin E[25] seem to increase the expression of LDL receptors in the cells, increasing cholesterol uptake by the liver cells and reducing the cholesterol plasma levels.

The serum and liver levels of the triacylglycerols, on the other hand, were not influenced by the treatments, a result similar to those found by Yang and Koo[20], in hypercholesterolemic rats fed green tea polyphenols. In the present study, a significant increase was observed in the fecal excretion of triacylglycerol and cholesterol. This effect could have been attributed to the interaction between polyphenols and the digestive proteins, altering lipid metabolism and reducing the bioavailability of these substances[26]. Polyphenolic substances can have an anti nutritional action compromising intestinal permeability and, consequently, animal nutrition[27], as well as their interaction with the pancreatic lipases, decreasing the action of the enzyme and compromising the bioavailability of the lipids[28].

Antioxidant capacity would be related to the modulation of oxidative stress. The progression of the atherosclerotic lesion involves an inflammatory process, where chemokines controls the recruitment of leukocytes within the vascular wall and these activated cells contribute to oxidative stress[29]. Increased serum levels of vitamin E of the groups treated reflects the enhancement of antioxidant capacity. The groups fed grape polyphenols were found to obtain a similar result regarding vitamin E serum levels to that obtained with α-tocopherol supplementation or even superior when the two antioxidants were administered. Such result may have been due to the action of α-tocopherol recovery by the polyphenols[30]. However, the results found regarding vitamin E levels in the liver suggest that the presence of polyphenols in the diet could mobilize liver vitamin reserves.

Polyphenols bioavailability determines their effects. Indirect evidence of their absorption through the gut barrier is the increase in the antioxidant capacity of the plasma with polyphenol-rich foods[18]. Bub *et al.*[31] administered fruit juices providing polyphenolic compounds to men during 2 weeks. Twelve hours after the last ingestion of juices no polyphenols or polyphenol metabolites from either juices were detected in plasma or urine samples, but positive results were found in the group supplemented. In our study, although no difference in plasma and liver polyphenol levels were found between the groups, animals receiving grape extract presented fecal content of polyphenols smaller than control and tocopherol group. This could indicate that supplemented animals absorbed polyphenol from the juice or may have been due to the interaction of the polyphenols with the enteric enzymes. The plasma levels tend to be constant, even during supplementation, when the polyphenol urinary excretion levels seem to increase proportionally to intake increase[20].

Although not presenting any statistical difference, the indicators of peroxidation in the present study tended to reduce these indices in the treatment using both antioxidants. These results were similar to those found by Stoker and O'Halloran[32], where no differences were observed in lipid peroxidation of ApoE-/-mice treated for 24 weeks with non-alcoholic red wine. Slight increase was observed in the formation of hydrogen peroxides, as in the present study, but with no statistical significance. Kaplan *et al.*[33] observed a reduction of 8% in lipid peroxidation in the plasma of Apo E-/- mice fed pomegranate juice, rich in polyphenols. Blackhurst and Marais[34] in a study with eutrophic humans did not observe any alteration in lipid peroxidation after red wine intake, although there was a significant increase in the polyphenol plasma levels. Zhao *et al.*[35] observed a reduction in lipid peroxidation in Apo E-/- mice fed diet supplemented with vitamin E

Fatty acid change can alter the properties of the cellular membranes and, consequently, their participation in the lipid metabolism[36]. The liver fatty acid profiles were not influenced by the treatments; however, despite non-significant, a greater preservation of the polyunsaturated fatty acids was observed in the polyphenol-fed groups (greater preservation of oleic acid in groups treated in comparison to control group). Such result may be an expression of solubility of the antioxidants studied. Hydrophilic phenolic extracts, sequesters of free radicals such as polyphenols, would be more effective antioxidants than the hydrophobic ones, such as vitamin E, in systems where there is an oil-water interface, such as the one found in the liver tissue[37].

Treatment using antioxidants was not capable of altering lesion size; however, there was a change in the degree of evolution of the atherosclerotic lesion, a result similar to that found by Nakata and Maeda[38], where researchers studied food supplementation with vitamin C in Apo E-/ mice. In the same animal model, Peluzio *et al.*[21] observed a significant reduction in the size of the lesions and smaller development degree, after vitamin E supplementation of 400 UI/Kg of diet. Zhao *et al.*[35] observed a reduction in atherosclerosis progression following food supplementation of vitamin E, with 2000UI/Kg of diet.

The polyphenol study reveals a reduction in the progression

of the atherosclerotic plaques. Fuhman *et al.*[39] observed a reduction in the degree of development of the atherosclerotic lesion after consumption of ginger polyphenols without pre induction of atheromatous plaque. Aviran *et al.*[40] and Kaplan *et al.*[41]observed reduction in the size of atherosclerotic lesion after pomegranate juice intake. Oral supplementation with non-alcoholic wine [32] and caffeic acid [41] were also capable of reducing the development of the atherosclerotic lesion.

Conclusions

1. Intake of antioxidants, such as the polyphenols found in grape, and α-tocopherol had a beneficial effect on atherosclerosis, contributing to reduce the degree of evolution of the atherosclerotic lesion, reduction of serum and liver cholesterol levels, increase of cholesterol and triacylglycerol fecal excretion.

2. A synergistic effect between the polyphenols and α-tocopherol was observed, resulting in a greater beneficial effect on atherosclerosis than the isolated consumption of antioxidants.

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