Effects of Vitamin E and Gemfibrozil on lipid profiles, lipid peroxidation and antioxidant status in the elderly and young hyperlipidemic subjects

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ABSTRACT

Objectives: This study has dealt with the effects of gemfibrozil and vitamin E (vit E) therapies on lipoprotein levels, lipid peroxidation and antioxidant statuses of the elderly and young hyperlipidemic subjects.

Methods: This study took place in the Internal Medicine Clinic, Faculty of Medicine, Osmangazi University, Turkey between 2004-2005. This study was carried out on 99 hyperlipidemic and 40 control subjects. Subjects were divided into 2 groups; elderly hyperlipidemic (n=65) and young hyperlipidemic (n=34). In the young and elderly hyperlipidemic subjects of the first group treated only with vit E (600 mg/day) for one month. In the young and elderly hyperlipidemic subjects of the second group were treated only with gemfibrozil (600 mg/twice daily) for one month. The 2 therapies of vit E and gemfibrozil were then combined and applied to the third group of our study. Reduced glutathione (GSH), glutathione peroxidase (GPx), total cholesterol (total chol), serum low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), vit E, malondialdehyde (MDA), superoxide dismutase (SOD) levels of the 3 groups were measured.

Results: In elderly hyperlipidemic therapy group: vit E groups, the post-treatment vit E levels increased. In the gemfibrozil groups, post-treatment TG level decreased whereas HDL level increased. In the vit E plus gemfibrozil groups, post-treatment TG level decreased, HDL, and vit E levels increased. In young hyperlipidemic therapy group: vit E groups, the post-treatment HDL, vit E, GSH, GPX levels increased whereas LDL, MDA, levels decreased. In the gemfibrozil groups, post-treatment TG, LDL decreased, HDL level increased. In the vit E plus gemfibrozil groups, post-treatment TG, LDL, MDA levels decreased whereas HDL, vit E, GSH levels increased.

Conclusion: When combined, gemfibrozil and vit E are effective in preventing cardiovascular diseases.

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Aging and hypercholesterolemia play an important role in atherosclerosis, and cardiovascular diseases related to it. Compared with the elderly, hypercholesterolemia is more serious in younger subjects, leading to premature atherosclerosis.[1,2] Endothelial cell damage is the basic mechanism for the initiation and maintenance of atherosclerosis. There is a strong evidence that hypercholesterolemia increases the production of reactive oxygen species (ROS) and leads to endothelial cell injury, which sets the stage for

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atherosclerosis. Reactive oxygen species have been implicated in tissue injury and various cardiovascular diseases (CVD).[3,4] Oxygen free radicals (OFRs) have been suggested to exert their cytotoxic effects by causing peroxidation of membrane phospholipids. This increases membrane fluidity, membrane permeability and the loss of membrane integrity.[3,5] End products produced during the lipid peroxidation process, including malondialdehyde (MDA), are very reactive and capable of cross-linking membrane proteins containing amino groups.[6] Gokkusu et al[7] have shown that MDA concentrations of plasma and liver homogenates increased in atherosclerosis. The evolution of oxidative processes within mammalian cells has necessitated the concomitant development of defensive mechanisms to prevent vital cell components against oxidative stress. Oxidative stress, the disturbance of the delicate balance between oxidants and antioxidants, may result from increased productions of free radicals and impaired antioxidant defense systems.[8,9] Vitamin E (Vit E) is an important lipid-soluble antioxidant placed in a special region of membranes. Its most important role is to protect the membrane polyunsaturated fatty acids from oxidation involving reactive oxygen species, by termination of free radical chain reactions.[8,9] Deprivation of dietary Vit E results in species-dependent and tissue-specific pathological lesions.[10] Various studies carried out on both experimental animals and human subjects have demonstrated that there is a negative correlation between cholesterol and Vit E concentrations, and that Vit E supplementation to diet reduces the elevated serum cholesterol concentrations.[11,12] A lipid-regulating agent that lowers elevated serum lipids primarily by decreasing serum triglycerides (TG) with a variable reduction in total cholesterol (TC). These decrease occur primary in VLD fraction and less frequently in low density lipoprotein (LDL) fraction. Gemfibrozil increases high density lipoprotein (HDL) subfraction HDL2 and HDL3 as well as apolipoproteins A-I and A-II.[13,14] Of the various treatment models that have so far been investigated to prevent the complication of atherosclerosis, lipid lowering drug and antioxidant treatments seem to be more promising than others.[15,16] In this study, the effects of lipid lowering drug therapy, Vit E therapy and application of both on lipoprotein levels, lipid peroxidation and antioxidant status were investigated in elderly and young hyperlipidemic subjects.

Methods. This study was carried out on 99 hyperlipidemic subjects who attended the Internal Medicine Clinic of our hospital between 2004 and 2005. These hyperlipidemic subjects were divided into 2 groups according to their ages: 1) Elderly hyperlipidemic group (71±2.9 years, n=65) and 2) the young hyperlipidemic group (29±4.39 years, n=34).

The exclusion criteria were as follows: acute illness or severe chronic disease, diabetes, hypertension, angina pectoris or previous myocardial infarction or peripheral vascular disease, thyroid dysfunction, alcohol intake, smoking, hormonal treatment, lipid-lowering medication, or vitamin or iron supplementation in the last 6 months before admission. Informed consents were obtained before the start of the study and the study protocol was approved by the Ethical Committee of the Osmangazi University Hospital. The elderly and young hyperlipidemic subjects were divided into 3 groups. For a period of one months, 20 elderly and 12 young hyperlipidemic subjects received Vit E (600mg/day)[17] orally; 23 elderly and 10 young hyperlipidemic subjects received gemfibrozil (600 mg twice daily);[16] 22 elderly and 12 young hyperlipidemic subjects received vitamin E (600 mg/day) + gemfibrozil (1200 mg/day). The body mass index (BMI) [wt (kg)/ht (m^2)] was obtained from measured height and weight before and after the therapy. Blood samples were collected from all subjects into heparinized and normal tubes both at the beginning and at the end of the one month of the therapy. Glutathione was measured in whole blood. Samples were immediately centrifuged at 1500 g for 5 min. Plasma and serum samples were isolated. Malondialdehyde levels were determined in plasma samples. Vitamin E, TC, TG, HDL, and LDL were measured in serum samples. After separating the plasma, erythrocytes were washed 3 times with saline and erythrocytes pellets prepared. Erythrocytes hemolysates were then prepared and stored at -25˚C until being assay for GPx and SOD activities. Serum TC, TG, and HDL were measured by means of Boehringer Mannheim Hitachi 742 autoanalyzer, using Boehringer Mannheim kits and enzymatic methods. Low density lipoprotein levels were calculated according to the following formula: LDL = TC-HDL cholesterol-TG/5. Serum vitamin E levels were determined according to the Hashim's method.[18] Lipid peroxidation assayed by the measurement of MDA levels on the base of MDA was reacted with thiobarbituric acid at 532 nm, according to Ohkawa et al.[19] Glutathione levels were measured according to Beutler.[20] Glutathione was reacted with 5.5 dithiobis-2-nitrobenzoic acid (DTNB) resulting in the formation of a product which has a maximal absorbency at 412 nm. Superoxide dismutase activity was determined according to Winterbourn et al.[21] One unit of SOD was designed as the amount of hemoglobin that inhibits the rate nitroblue tetrazolium (NBT) reduction by

50%. Glutathione peroxidase activity was determined spectrophotometrically at 340 nm according to Paglia and Valentine.[22] The reaction mixture consisted of phosphate buffer (pH: 7), ethylenediaminetetraaceti c acid (EDTA), glutamate or sodium azide (NaN_3), triphosphopyridine nucleotide (NADPH), glutathione reductase GSH and H_2O_2. All ingredients except the enzyme source and H_2O_2 were combined at the beginning of each day. Blank reactions with the enzyme source replaced by distilled water were subtracted from each assay for non-enzymatic oxidation of NADPH by the peroxides. All the chemicals were obtained from Sigma Chemical Company (USA).

Data were given as a mean ±SEM. Significant differences were established using student's t-test. A probability value of less than 0.05 was taken as significant.

Results. The data of mean age, systolic and diastolic blood pressure and BMI of the 3 groups are shown in **Table 1**. No statistically significant differences have been found between the subjects in terms of demographic characteristics.

Elderly hyperlipidemic group. Total-cholesterol, lipoproteins and Vit E (**Table 2**), MDA, GSH, GPx and SOD levels (**Table 3**) were determined in 65 elderly hyperlipidemic subjects and 20 healthy cases. Total-cholesterol, TG, LDL-chol, MDA levels in elderly hyperlipidemic group were higher than in the control group. However, HDL-chol, Vit E , GPx and SOD levels decreased. In the Vit E group, post-treatment Vit E levels increased. No significant difference was found in the levels of total-chol, TG, HDL-chol, LDL-chol, MDA, GSH, GPx and SOD. In the gemfibrozil group, post-treatment TG

Table 1 - Clinical characteristics of hyperlipidemic elderly and young groups.

Clinical characteristics	Elderly hyperlipidemic			Young hyperlipidemic		
	Vitamin E n=20	Gemfibrozil n=23	Vitamin E plus Gemfibrozil n=22	Vitamin E n=12	Gemfibrozil n=10	Vitamin E plus Gemfibrozil n=12
Age (years)	70 ± 1.2	71 ± 3	72 ± 4.2	27 ± 3.2	29 ± 5.3	30 ± 4.6
Gender (M/F)	12/8	13/10	11/11	7/5	5/5	6/6
Body mass index (kg/m^2)	26 ± 4.9	26 ± 6.0	25 ± 3.4	25 ±2 .6	24 ± 2.8	23 ± 3.2
Body weight (kg) t=0	69 ± 8.4	67 ± 6.0	63 ± 4.7	64 ± 6.9	64 ± 5.5	62 ± 4.7
Body weight (kg) t=4	67 ± 7.2	66 ± 3.8	60 ± 8.2	62 ± 5.2	63 ± 6.1	60 ± 7.1
Systolic blood pressure (mm Hg)*	135 ± 3.0	130 ± 5.0	136 ± 7.0	130 ± 2.7	129 ± 8.5	130 ± 6.6
Systolic blood pressure (mm Hg)†	130 ± 2.8	125 ± 6.7	132 ± 8.0	127 ± 6.4	128 ± 7.7	125 ± 5.0
Diastolic blood pressure (mm Hg)*	88 ± 1.5	84 ± 3.4	86 ± 6.7	85 ± 2.4	83 ± 5.0	80 ± 3.7
Diastolic blood pressure (mm Hg)†	85 ± 2.7	80 ± 6.4	84 ± 4.0	80 ± 6.2	81 ± 4.2	80 ± 3.9

M/F - male/female, *zero week, †4 weeks

Table 2 - Lipid profiles in the control and elderly hyperlipidemic groups.

Groups	Total cholesterol (mmol l^{-1})	Triglyceride (mmol l^{-1})	HDL (mmol l^{-1})	LDL (mmol l^{-1})	Vitamin E (μmol/L)
Control (n=20)	4.14 ± 1.2	1.24 ± 0.2	1.29 ± 0.03	2.61 ± 0.5	31.7 ± 1.8
Hyperlipidemic (n=65)	7.6 ± 2.2	3.6 ± 1.02	0.49 ± 0.02	6.45 ± 1.8	19.03 ± 2.0
p-value	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.01
Vitamin E (n=20)					
Before	7.7 ± 1.08	3.5 ± 0.08	0.51 ± 0.04	6.49 ± 1.8	19.4 ± 1.7
After	7.2 ± 1.2	3.4 ± 0.07	0.69 ± 0.02	5.91 ± 1.05	27.8 ± 2.3
p-value	NS	NS	NS	NS	*p*<0.05
Gemfibrozil (n=23)					
Before	7.5 ± 1.9	3.7 ± 1.3	0.47 ± 0.3	6.29 ± 1.05	20 ± 1.15
After	7.1 ± 2.3	2.5 ± 1.7	0.82 ± 0.04	5.81 ± 1.3	22 ± 2.03
p-value	NS	*p*<0.001	*p*<0.05	NS	NS
Vitamin E + Gemfibrozil (n=22)					
Before	7.6 ± 1.18	3.6 ± 0.18	0.49 ± 0.3	6.39 ± 2.0	19 ± 0.06
After	7.3 ± 1.07	2.23 ± 0.07	0.82 ± 0.04	5.99 ± 1.22	26 ± 0.1
p-value	NS	*p*<0.001	*p*<0.05	NS	*p*<0.05

Means±SEM, HDL - high density lipoprotein, LDL - low density lipoprotein

Table 3 - Malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GPx), levels in the control, elderly hyperlipidemic, Vitamin E, gemfibrozil and Vitamin E + Gemfibrozil given as mean±SEM.

Groups	MDA (nmol/ml)	GSH (mg/dl Eryth)	GPX (U/g Hb)	SOD (U/g Hb)
Control (n=20)	3.4 ± 0.5	102 ± 3.8	15.6 ± 0.4	2436 ± 45.6
Hyperlipidemic (n=65)	5.6 ± 0.02	76 ± 2.7	11.0 ± 0.7	2118 ± 66.9
P-value	*p*<0.001	*p*<0.01	*p*<0.001	*p*<0.001
Vitamin E (n=20)				
Before	5.5 ± 1.0	78 ± 3.0	11.8 ± 0.18	21230 ± 78.7
After	5.0 ± 0.7	84 ± 2.16	12.0 ± 0.17	2153 ± 74.3
P-value	Not significant	Not significant	Not significant	Not significant
Gemfibrozil (n=3)				
Before	5.6 ± 0.07	77 ± 2.8	10.7 ± 0.5	2125 ± 55.8
After	5.4 ± 0.45	86 ± 2.25	11.5 ± 0.4	2145 ± 60.4
P-value	Not significant	Not significant	Not significant	Not significant
Vitamin E + Gemfibrozil (n=22)				
Before	5.7 ± 0.8	75 ± 4.0	11.2 ± 0.2	2116 ± 70.5
After	5.3 ± 0.2	82 ± 3.14	11.9 ± 0.25	2130 ±72.9
P-value	Not significant	Not significant	Not significant	Not significant

Table 4 - Lipid profiles in the control and young hyperlipidemic groups (mean±SEM).

Groups	Total cholesterol (mmol l^{-1})	Triglyceride (mmol l^{-1})	HDL (mmol l^{-1})	LDL (mmol l^{-1})	Vitamin E (μmol/L)
Control (n=20)	4.6 ± 0.08	1.02 ± 0.03	1.68 ± 0.08	2.65 ± 0.15	43.4±0.08
Hyperlipidemic (n=34)	7.3 ± 0.1	3.12 ± 0.07	0.77 ± 0.05	5.93 ± 0.22	38.6±0.05
P-value	*p*<0.001	*p*<0.001	*p*<0.01	*p*<0.01	Not significant
Vitamin E (n=12)					
Before	7.4 ± 0.07	3.05 ± 0.12	0.80 ± 0.05	5.82 ± 0.06	37.3±0.07
After	6.3 ± 0.03	2.71 ± 0.08	1.38 ± 0.13	4.70 ± 0.08	46.6±0.06
P-value	Not significant	Not significant	*p*<0.05	*p*<0.05	p<0.05
Gemfibrozil (n=10)					
Before	7.25 ± 0.15	3.11 ± 0.05	0.75 ± 0.04	5.91 ± 0.05	35.3±0.04
After	6.55 ± 0.07	1.97 ± 0.18	1.41 ± 0.08	4.72 ± 0.1	30±0.01
P-value	Not significant	*p*<0.001	*p*<0.05	*p*<0.05	Not significant
Vitamin E + Gemfibrozil (n=12)					
Before	7.35 ± 0.04	3.15 ± 0.04	0.76 ± 0.06	5.98 ± 0.18	36±0.03
After	6.58 ± 0.18	1.88 ± 0.07	1.57 ± 0.04	4.61 ± 0.12	45.9±0.02
P-value	Not significant	*p*<0.001	*p*<0.01	*p*<0.01	*p*<0.05

Table 5 - Malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GPx), levels in the control, young hyperlipidemic, Vitamin E, gemfibrozil and Vitamin E + Gemfibrozil given as Mean±SEM.

Groups	MDA (nmol/ml)	GSH (mg/dl Eryth)	GPX (U/g Hb)	SOD (U/g HB)
Control (n=20)	2.3 ± 0.07	126 ± 3.4	8.7 ± 0.4	3131 ± 58.7
Hyperlipidemic (n=34)	3.28 ± 0.06	105 ± 2.6	5.2 ± 6.3	3115 ± 6.8
P-value	*p*<0.01	*p*<0.01	*p*<0.05	Not significant
Vitamin E (n=12)				
Before	3.31 ± 0.05	106 ± 1.8	5.0 ± 0.5	3120 ± 68.7
After	1.96 ± 0.02	123 ± 3.0	7.9 ± 0.4	3110 ± 74.6
P-value	*p*<0.05	*p*<0.05	*p*<0.01	Not significant
Gemfibrozil (n=10)				
Before	3.25 ± 0.02	105 ± 2.9	5.2 ± 0.3	3128 ± 55.7
After	3.00 ± 0.03	112 ± 3.0	5.8 ± 0.4	3100 ± 60.6
P-value	NS	NS	NS	Not significant
Vitamin E ± Gemfibrozil (n=12)				
Before	3.29 ± 0.02	104 ± 2.3	5.3 ± 0.2	3110 ± 70.7
After	1.92 ± 0.04	121 ± 1.7	8.1 ± 0.4	3100 ± 72.0
P-value	*p*<0.05	*p*<0.01	*p*<0.05	Not significant

level decreased whereas HDL-chol level increased. No significant differences was found in the levels of total-chol, LDL-chol, MDA, GSH, GPx and SOD. In the Vit E + gemfibrozil group, post-treatment TG level decreased whereas HDL-chol and Vit E levels increased. No significant differences was found in the levels of total-chol, LDL-chol, MDA, GSH, GPx and SOD.

Young hyperlipidemic group. Total-chol, lipoproteins and Vit E (**Table 4**), MDA, GSH, GPx and SOD levels (**Table 5**) were determined in 34 young hyperlipidemic subjects and 20 healthy cases. Total-chol, TG, LDL-chol, MDA levels in young hyperlipidemic group were higher than in the control group. However, HDL-chol, GSH and GPx levels decreased. Vitamin E and SOD levels were not different. In the Vit E group, post-treatment Vit E, HDL-chol, GSH, GPx levels increased. However, LDL-chol, MDA levels decreased. No significant differences was found in the levels of total-chol, TG and SOD. In the gemfibrozil group, post-treatment TG, LDL-chol levels decreased. Whereas HDL-chol level increased. No significant differences was found in the levels of total-chol, Vit E, MDA, GSH, GPx and SOD. In the gemfibrozil + Vit E group, pre-treatment levels of TG, LDL-chol and MDA were high compared to post-treatment. Inversely HDL-chol, Vit E, GSH and GPx levels increased after treatment. However, there was no significant difference in the levels of total-chol and SOD.

Discussion. In this study, the effects of gemfibrozil therapy, Vit E therapy and combined therapy of both on lipoprotein levels, lipid peroxidation and antioxidant statuses have been investigated in the elderly and young hyperlipidemic subjects. Vitamin E, a lipophilic compound acting as a chain breaking antioxidant, has been shown to prevent lipid peroxidation, especially in lipid phases.[9,23,24] A stabilizing role of Vit E for phospholipids in membranes has also been reported.[25] While some studies suggest that Vit E supplementation to diet might prevent serum lipids from increasing.[3,9,23] There are others to be the contrary.[11,12] A number of studies have investigated the inhibition of atherosclerosis in a quantitative manner of Vit E in hypercholesterolemic animals. Rabbits were used in most of these studies. Viswanathan et al[24] reported an increase in serum TG, a decrease in LDL and no change in serum total cholesterol in rabbits treated with Vit E. Komaratat et al[26] observed a decrease in total-chol, LDL and verly low density lipoprotein (VLDL) and an increase in HDL with high doses of vitamin E in rabbits. In another study, Prasad and Kalra,[3] reported that 0.04 g/kg Vit E administration did not change the lipid profile in rabbit plasma. These controversial results could be due to the varying does of Vit E used. Rezaian et al[27] reported that Vit E could effectively lower the serum cholesterol and LDL levels and rise the serum HDL level in the middle aged to elderly healthy individuals. Several such studies reported a reduction in relative risk of clinical cardiovascular disease,[27,28] cardiovascular disease mortality,[29] angiographically detected progression of coronary[30] or carotid[31] atherosclerosis as assessed by ultrasound[25] in association with increased intake of Vit E from food or supplements. Among these studies, there is a general agreement that the minimum level of supplementation associated with benefit is about 100 IU.[27,28,30,31] Two studies have reported that the association of increased intake of Vit E from supplements with reduced risk of coronary heart disease was strongest for those who had taken the supplements for more than 2 years.[27,28] The results achieved by Qiao et al[32] were similar to those of the above-mentioned studies. In this study, concentrations of serum lipid profiles in the elderly hyperlipidemic group seemed not to be affected by Vit E treatment. However, in the young hyperlipidemic group treated with Vit E, the use of this vitamin caused a decline in LDL, TG and an increase in HDL. Vitamin E can inhibit the production of oxidatively modified LDL, which suggests that it may play a protective role in atherogenesis.[10,33] Hypercholesterolemia-induced atherosclerosis and endothelial impairment by free radical formation shown markedly suppressed after treatment with vitamin E. Prasad[3] reported a decrease in the blood MDA concentrations in rabbits supplemented with Vit E. On the other hand, Szezeklit et al[34] observed no change in plasma MDA concentrations in Vit E supplemented and cholesterol-fed rabbits.[34] Our findings indicate that plasma MDA concentrations, as a marker of oxidative stress, decrease when young hyperlipidemic cases are supplemented with Vit E. The preventive effect of Vit E on the MDA concentration in hyperlipidemic subjects may be related to the trapping of the chain-propagating peroxyl radicals. Protein thiolase are physiologically free radical scavengers and may serve as antioxidants by several mechanisms. They may preemptively scavenge oxidants that initiate peroxidation, thus sparing Vit E and lipids from attack of peroxidation.[35,36] As a marker of free radical scavenging, GSH may be a primary agent involved in redox regulation of protein thiols.[37] In our study, increases in the GSH contents in the blood were observed in young hyperlipidemic cases administrated Vit E. It was also observed that Vit E supplementation caused the activation of GSH-related enzyme. All of these results indicate that high

level of cholesterol causes important changes in the relations between tissue oxidant-antioxidant balance and lipid parameters, and Vit E supplementation partly decrease these relations. Our data indicate that MDA, GSH, GPx levels in elderly hyperlipidemic subjects seemed not to be affected by Vit E treatment. In order for our group to explain in more detail, the reasons why Vit E was not effective, more research is needed. Gemfibrozil is a fibric acid derivative which specifically lowers lipid and cholesterol concentrations. In some studies have reported a decrease in serum TG, LDL, and an increase in HDL in the hyperlipidemic cases treated with gemfibrozil.[38,39] Our results are in agreement with those of Aberg[16] and Vasquez.[40] The main effect of gemfibrozil is on LDL in the young hyperlipidemic subjects. In neither the elderly nor the young hyperlipidemic groups were any changes observed in the levels of TC, Vit E, MDA, GSH, GPx or SOD. Likewise, following combined therapy, no significant changes were found in the TC, LDL, MDA, GSH, GPx, or SOD levels of the elderly hyperlipidemic subjects. In both the elderly and young hyperlipidemic group receiving combined therapy the rise in the post-treatment level of HDL and the decline in that of TG were noteworthy. This allows us to suggest that the post-treatment rise in the HDL and decline in the level of TG were caused by the gemfibrozil therapy. In the young hyperlipidemic subjects of the group treated with gemfibrozil+Vit E, Vit E appears to have caused significant redistribution of cholesterol among the lipoproteins by decreasing the fraction of LDL and increasing that of HDL. Hyperlipidemia causes reduction in the enzymatic antioxidant defense potential of tissues and leads to oxidant stress due to both this reduction in antioxidant capacity and free radical load caused by the high level of cholesterol. As a result of these metabolic events peroxidation reactions are accelerated and some important changes occur in plasma lipid parameters. Administration of Vit E may active antioxidant enzymes and scavenge toxic free radicals thus may protect cellular structures against peroxidation reactions caused by hypercholesterolemia.

In conclusion, our results suggest that in order to prevent atherosclerosis in hyperlipidemic subjects, gemfibrozil plus Vit E therapy may be more effective than gemfibrozil therapy alone.

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