# Effects of policosanol treatment on the susceptibility of low density lipoprotein (LDL) isolated from healthy volunteers to oxidative modification *in vitro*

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***Aims*** The aim of this study was to investigate the effect of policosanol on the susceptibility of LDL-C to *in vitro* lipid peroxidation in human healthy volunteers.
***Methods*** The effect of policosanol (5 and 10 mg $day^{-1}$) on LDL-C oxidation was studied in a double-blind, randomized, placebo-controlled trial conducted in 69 subjects. LDL-C samples isolated at baseline and after 8 weeks were subjected to *in vitro* tests of LDL-C oxidation. We tested the susceptibility of LDL-C to lipid peroxidation in a cell-free system by the addition of copper ions as well as in a more physiological system, macrophage-mediated oxidation.
***Results*** At baseline all groups were well matched regarding all variables. After 8 weeks of therapy policosanol administered at 5 and 10 mg, significantly and in a dose-dependent manner increased the lag phase of conjugated diene generation (mean $\pm$ s.d.) from $83.79 \pm 29.16$ min to $94.90 \pm 25.50$ min (5 mg $day^{-1}$) and from $82.74 \pm 17.16$ min to $129.89 \pm 35.71$ min (10 mg $day^{-1}$), while in the placebo group LDL-C oxidation did not change significantly. Policosanol (10 mg $day^{-1}$), but not placebo, significantly decreased the rate of conjugated diene generation. Comparison with placebo after therapy also showed significant differences. Macrophage mediated-oxidation was also inhibited by policosanol as evident by measuring thiobarbituric acid reactive substances (TBARS). Policosanol (10 mg $day^{-1}$) significantly lowered malondialdehyde (MDA) generation from $8.50 \pm 0.91$ to $5.76 \pm 1.01$ nmol $mg^{-1}$ protein. Comparison with placebo after 5 and 10 mg $day^{-1}$ showed significant differences. Policosanol significantly lowered total cholesterol by 10.5% (5 mg $day^{-1}$) and 12.4% (10 mg $day^{-1}$) and LDL-C by 16.7% and 20.2%, respectively. Also, policosanol (10 mg $day^{-1}$) increased HDL-C by 15.2%. Five subjects withdrew from the study, none because of adverse experiences. No clinical or blood biochemical drug-related disturbances were found.
***Conclusions*** The present study demonstrated that policosanol administered within its therapeutic dosage for lowering cholesterol (5 and 10 mg $day^{-1}$), decreased the susceptibility of LDL-C to lipid peroxidation *in vitro*.

*Keywords:* copper-mediated oxidation, healthy volunteers, LDL lipid peroxidation, macrophage-mediated oxidation, policosanol

## Introduction

The relation between coronary heart disease (CHD) and elevated concentrations of serum total cholesterol, particularly low density lipoprotein cholesterol (LDL-C) has been demonstrated repeatedly [1–3] and the beneficial effect of drug therapy for lowering cholesterol in

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hypercholesterolaemic patients had also been proved convincingly [1, 4]. Several lines of evidence indicate that the oxidative modification of LDL-C may provide an important link between plasma LDL-C and the development of atherosclerosis [5, 6]. Oxidation of LDL-C is a lipid peroxidation process in which the unsaturated fatty acids (PUFAS) contained in the LDL-C are transformed into lipid hydroperoxides and then to some unsaturated aldehydes. These products formed within the LDL-C particles, are believed to bind covalently with the amino acid side chain of apo B, modifying its positively charged amino acid residues. This reduces the apo B affinity to the LDL-C receptor and increases its affinity to the scavenger receptor [7]. The oxidized LDL-C is taken up by macrophages leading to formation of lipid-laden foam cells, the hallmark of early atherosclerotic lesion [8].

Other properties of the modified LDL-C (oLDL-C) may increase its atherogenicity. Modified LDL-C also may promote atherosclerosis by its chemotactic properties to monocytes [9], its cytotoxicity to arterial wall endothelial cell [10] and its inhibition of nitric oxide-mediated vasodilation [11]. Thus, the array of atherogenic properties of oLDL-C makes the prevention of its formation an attractive strategy to prevent coronary heart disease in humans.

Policosanol is a mixture of higher primary aliphatic alcohols purified from sugar cane wax with cholesterol-lowering effects, demonstrated in animals models [12, 13], healthy volunteers [14] and patients with type II hypercholesterolaemia [15–29]. Preclinical studies have demonstrated that policosanol administered orally for 4 weeks to normocholesterolaemic rats, partially prevented *in vitro* and *in vivo* microsomal lipid peroxidation [30] and inhibited lipid peroxidation of lipoprotein fractions containing LDL-C + VLDL-C [31]. Policosanol significantly prolonged the lag phase of oxidation, decreased the propagation phase and diminished the maximal amount of diene generation. Also, policosanol significantly decreased malondialdehyde (MDA) formation in LDL-C + VLDL-C fractions and attenuated the reduction in the reactivity of lysine amino groups of the lipoprotein after copper oxidation, which could be related to a protection of the protein moiety of lipoproteins.

The present study was aimed to investigate the effect of policosanol administered within its usual dose range as a cholesterol-lowering drug, on the susceptibility of LDL-C to *in vitro* lipid peroxidation in human healthy volunteers.

## Methods

A total of 69 normocholesterolaemic (serum cholesterol levels $<5.2$ mmol $l^{-1}$ and triglycerides $<2.2$ mmol $l^{-1}$) of both sexes aged 20–60 years were included in this study. Subjects who were current smokers, abusers of alcohol, with any chronic pathology as well as those with acute diseases at the recruitment time were excluded from the study. In addition, subjects with documented consumption of antioxidant supplements or vitamins within the last 3 months prior to the study and pregnant women were also excluded. Consumption of any drug or supplement with recognized antioxidant effect was not allowed during the study.

### *Study design*

The present study was performed in accordance with the Declaration of Helsinki and was approved by the ethic committee of the National Center for Scientific Research. The subjects were enrolled after giving written informed consent. At this time, general data were recorded and clinical examination was performed. Subjects who met the study entry criteria were allocated randomly, under double-blind conditions, to received either placebo

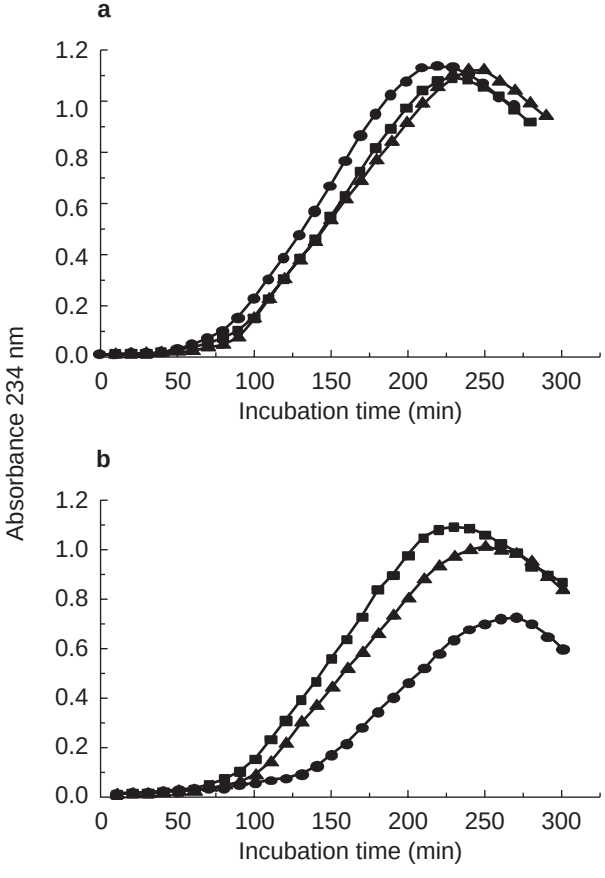


**Figure 1.** Formation of conjugated dienes during oxidation of LDL-C a) before and b) after policosanol and placebo treatment. LDL-C (50 $\mu$g $ml^{-1}$) was incubated at 37°C in PBS containing 5 $\mu$M $Cu^{2+}$. Changes in absorbance at 234 nm were measured every 10 min for 5 h. The data show results for (■) placebo group and policosanol treated groups: (▲) 5 mg $day^{-1}$ and (●) 10 mg $day^{-1}$.


 

($n=23$), policosanol 5 mg ($n=24$) or policosanol 10 mg ($n=22$). Volunteers were instructed to take the tablets once a day with the evening meal for 8 weeks. Lipid peroxidation, blood tests, clinical examination and request for adverse experiences were performed at baseline and repeated after 8 weeks of therapy.

Blood samples were taken after 12 h overnight fasting to determine lipid profile, blood biochemistry safety indicators and to assay lipid peroxidation. Total serum cholesterol, triglycerides and blood chemistry parameters (glucose, ASAT, ALAT) were assayed by enzymatic methods using reagent kits from Boehringer Mannhein (Mannhein, Germany). Levels of high density lipoprotein cholesterol (HDL-C) were determined as the cholesterol content on the supernatant obtained after β-lipoprotein precipitation [32]. LDL-C was calculated using the Friedewald formula [33]. Laboratory analyses were carried out in a Hitachi autoanalyser at the Medical Surgical Research Center. Systematic quality control was performed throughout the study.

### *Techniques to assess LDL-C oxidation*

*Isolation of LDL-C.* After an overnight fast, venous blood samples were collected into plastic tubes containing the appropriate volume of an aqueous solution of 10% EDTA to obtain a final blood concentration of 0.1% EDTA (w/v). Plasma was separated immediately after collection by centrifugation, supplemented with saccharose (final concentration 6 g $l^{-1}$) to prevent LDL-C aggregation and stored at $-80^{\circ}$C [34]. Samples were stored for up to 3 weeks. Frozen EDTA-plasma were rapidly thawed and used for preparation of LDL-C by density-gradient ultracentrifugation (22 h at 285 000 *g* (max) in a Beckman SW40 rotor in a Beckman L7 ultracentrifuge), according to Terpsta *et al.* [35] without prestaining with amido black. The isolated LDL-C was dialysed against a 200-fold volume of phosphate-buffered saline (PBS, 0.01 M phosphate, 0.15 NaCl, pH 7.4) at 4°C in the dark for 24 h. The buffer was changed twice during this time and purged with nitrogen before use.

LDL-C concentration was estimated by determining protein concentration by a modification of the Lowry procedure [36].

*Kinetics of $Cu^{2+}$ induced oxidation of LDL-C.* Following overnight dialysis LDL-C (50 μg protein $ml^{-1}$) was oxidized in the presence of $CuSO_4$ 5 μM (final concentration). The kinetics of LDL-C oxidation was followed by continuously monitoring the change in absorbance at 234 nm in a Ultrospec plus spectrophotometer (LKB) equipped with a six-position automatic sample changer thermostat-controlled (37°C). Data derived from the conjugated diene curves were expressed as time course curves. The time profile of the absorption showed three distinct phases; (1) a lag phase during which absorption does not increase or increases only slightly, (2) a propagation phase, during which the absorbance rapidly increases to a maximum value and (3) the decomposition phase, in which after reaching the maximum value the conjugated dienes slowly decreases by decomposition to form aldehydes. From this kinetic a tangent was drawn to the steep segment of the curve of the propagation phase and extrapolated to the horizontal axis (time). The interval between the addition of $Cu^{2+}$ ions and the intersection point on this axis is defined as the lag time and is expressed in minutes. The propagation rate was calculated from the slope of the tangent of the curve during the propagation phase. Using a molar coefficient extinction for conjugated dienes of 29 500 $M^{-1}.cm^{-1}$ the rate of conjugate dienes generation is expressed in nmol of dienes formed $min^{-1}$ $mg^{-1}$ of LDL-C protein. Each LDL-C preparation was oxidized in triplicate. The reproducibility of the lag phase, was checked over a 3-week period. LDL-C was isolated from a plasma pool after different times of storage and repeated lag time measurements were made. The intra-

**Table 1** Baseline characteristics of study subjects (mean ± s.d.).

| | *Placebo* n = *23* | *Policosanol* *(5 mg)* n = *24* | *(10 mg)* n = *22* | | *Total* n = *69* |
|---|---|---|---|---|---|
| Age (years) | 40 ± 10 | 38 ± 10 | 40 ± 11 | NS | 39 ± 10 |
| Body weight (kg) | 64.2 ± 10.4 | 65.4 ± 11.5 | 67.9 ± 17.2 | NS | 65.8 ± 13.2 |
| Sex (M/F) | 9/14 | 11/13 | 8/14 | NS[1] | 28/14 |
| Total cholesterol (mmol $l^{-1}$) | 4.40 ± 0.66 | 4.60 ± 0.72 | 4.64 ± 0.55 | NS | |
| LDL-C | 2.99 ± 0.63 | 3.19 ± 0.67 | 2.65 ± 0.66 | NS | |
| Triglycerides | 1.21 ± 0.42 | 1.14 ± 0.49 | 1.06 ± 0.45 | NS | |
| HDL-C | 0.96 ± 0.25 | 0.98 ± 0.22 | 0.96 ± 0.22 | NS | |

(*n*) number of subjects; NS No significant difference from placebo (Mann Whitney *U*-test).
NS [1] Not significant compared with placebo (Fisher's Exact Test).

**Table 2** Lipid profile (mmol $l^{-1}$) in subjects treated with placebo or policosanol (mean ± s.e.mean results).

| *Group* | *Baseline* | *8 weeks* | *%* |
|---|---|---|---|
| *Total cholesterol* | | | |
| Placebo | 4.40 ± 0.66 | 4.57 ± 0.55 | +4.4 |
| Policosanol-5 | 4.60 ± 0.72 | 4.10 ± 0.63**** ++ | −10.5 ++++ |
| Policosanol-10 | 4.64 ± 0.58 | 4.06 ± 0.64 **** ++ | −12.4 ++++ |
| *LDL-C* | | | |
| Placebo | 2.99 ± 0.63 | 3.12 ± 0.51 | +7.3 |
| Policosanol-5 | 3.19 ± 0.67 | 2.65 ± 0.66 **** + | −16.7 ++++ |
| Policosanol-10 | 3.28 ± 0.63 | 2.64 ± 0.67 **** + | −20.2 ++++ |
| *HDL-C* | | | |
| Placebo | 0.96 ± 0.25 | 0.94 ± 0.21 | −1.8 |
| Policosanol-5 | 0.98 ± 0.22 | 1.07 ± 0.28 ** + | +9.0 + |
| Policosanol-10 | 0.96 ± 0.22 | 1.08 ± 0.21 ***$_+$ | +15.2 ++ |
| *Triglycerides* | | | |
| Placebo | 1.21 ± 0.42 | 1.37 ± 0.66 | +17.6 |
| Policosanol-5 | 1.14 ± 0.49 | 1.03 ± 0.49 + | −9.7 [b] |
| Policosanol-10 | 1.06 ± 0.45 | 0.90 ± 0.39 ** +[a] | −9.4 [b] |

% percent changes (–) decrease; (+) increase. ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$. Comparison with baseline (Wilcoxon Test for paired samples). [b] $P=0.05$, + $P<0.05$, ++ $P<0.01$; +++ + $P<0.0001$. Compared with placebo (Mann Whitney *U*-test), [a] significantly different for the level of significance $P<0.016$ (Bonferroni correction).

assay coefficient of variation for a single LDL-C solution was 2.48% and the interassay variability obtained in four different days gave a coefficient of variation of 3.88%. Before starting the trial, the stability of different plasma samples stored in our experimental conditions was verified using LDL-C isolated from five donors. The variability of lag phase measurements up to 3 weeks of storage was in the range of 3.0–5.8% (mean, 4.4%).

*Cell-mediated LDL-C oxidation.* After dialysis, EDTA-free LDL-C samples were immediately subjected to a cell-mediated oxidation by resident macrophages freshly isolated from male BalC mice according to de Whalley *et al*. [37]. Macrophages were isolated by peritoneal lavage of mice (20–30 g) using ice cold Dulbecco's phosphate buffered saline without $Ca^{2+}$ and $Mg^{2+}$. The cells were plated in 12-well cluster plates at $1 \times 10^6$ cells/well and incubated at 37°C in $CO_2$. The cultured medium consisted of 9 volumes of Dulbecco's modified Eagle's medium (DMEM) and 1 volumes of fetal calf serum contained 50 μg gentamicin $ml^{-1}$. After 2 h, the macrophages were washed several times with DMEM and were immediately used for experiments. LDL-C samples (100 μg $ml^{-1}$) were incubated up to 20 h with macrophages (macrophage-modified) or cell-free wells (controls). The cultured medium (0.5 ml $well^{-1}$) consisted of Ham's F-10 medium containing gentamicin (50 μg $ml^{-1}$). After incubation, EDTA and butylated hydroxytoluene were added (final concentration of 20 μM and 2 mM, respectively) to avoid any further oxidation and the medium was removed and centrifuged (250 *g* for 10 min at 4°C) to removed any detached cells. LDL-C in the medium was assayed for thiobarbituric acid reactive substances (TBARS) as described previously [38]. Freshly diluted malondialdehyde bis(dimethyl acetal) was used as a reference standard. TBARS were expressed as MDA equivalent content (nanomoles of MDA per milligram of protein). Each LDL-C preparation was oxidized in triplicate.

### *Statistical analysis*

Results are expressed as mean ± s.d.. Comparisons within groups were carried out by the Wilcoxon rank test for

**Table 3** Changes in kinetics of copper-mediated oxidation.

| *Groups* | *Baseline* | *8 weeks* |
|---|---|---|
| *Lag time (min)* | | |
| Placebo | 88.17 ± 23.38 | 89.22 ± 16.62 |
| Policosanol-5 | 83.79 ± 29.16 | 94.90 ± 25.50 ** |
| Policosanol-10 | 82.74 ± 17.16 | 129.89 ± 35.71 *** +++ |
| *Propagation rate (nmol diene $min^{-1}$ $mg^{-1}$ protein)* | | |
| Placebo | 11.26 ± 3.57 | 11.13 ± 2.09 |
| Policosanol-5 | 10.23 ± 2.24 | 9.06 ± 1.75 + NS |
| Policosanol-10 | 12.53 ± 3.56 | 7.80 ± 2.64 *** ++ |

** $P<0.01$; *** $P<0.001$. Comparison with baseline (Wilcoxon Test). + $P<0.05$, ++ $P<0.01$; +++ $P<0.001$. Comparison Vs Placebo (Mann Whitney *U*-test), NS, no significantly different for the level of significance $P<0.016$ (Bonferroni correction).



 

paired samples and the Mann Whitney *U*-test was used for comparisons between groups. A two-tailed probability value $<0.05$ was considered as statistically significant. Bonferroni adjustment was applied for multiple comparisons in a single test [39]. To adjust for multiple testing, $\alpha < 0.016$ $(0.05 \div 3)$ was chosen as the level of significance. Statistical analyses were performed using a CSS statistical program package (Star Soft, Tulsa, Oklahoma).

## Results

Table 1 shows the main baseline characteristics of the study subjects. All groups were well matched at baseline. Of the 69 subjects randomized to double-blind treatment, 64 completed the study.

Table 2 summarizes the effects of the treatments on lipid profile. At randomization lipid profile was similar in all the groups. Policosanol (5 and 10 mg day$^{-1}$) significantly lowered from baseline total cholesterol ($P<0.0001$) (10.5% and 12.4% respectively) and LDL-C ($P<0.0001$) (16.7% and 20.%, respectively). Policosanol (5 and 10 mg day$^{-1}$) increased HDL-C by 9.0% and 15.2%, respectively. These changes were statistically significant ($P<0.01$ for 5 mg day$^{-1}$ and $P<0.001$ for 10 mg day$^{-1}$). Also, policosanol (10 mg day$^{-1}$) lowered triglycerides by 9.4% ($P<0.01$). Besides, at 8 weeks of therapy, plasma LDL-C levels between the placebo group and policosanol (5 and 10 mg day$^{-1}$) decreased ($P=0.020$ and $P=0.019$, respectively. After 8 weeks of therapy with policosanol (10 mg day$^{-1}$) HDL-C increased ($P=0.03$). Final triglycerides levels decreased after policosanol 5 mg day$^{-1}$ ($P=0.04$) and 10 mg day$^{-1}$ ($P=0.011$). Bonferroni adjustment affected significance. Thus, comparison of final plasma LDL-C concentrations between policosanol (5 and 10 mg day$^{-1}$) and placebo and final plasma HDL-C concentrations between policosanol (10 mg day$^{-1}$) and placebo groups were not significantly different using Bonferroni correction ($P<0.016$). Similarly, triglycerides concentrations at 8 weeks between policosanol (5 mg day$^{-1}$) and placebo groups were not significantly different. Moreover, comparisons of percent changes between policosanol (5 and 10 mg day$^{-1}$) and placebo groups of total cholesterol and LDL-C after 8 weeks were highly significant ($P<0.0001$). For HDL-C levels, percent changes increased after policosanol 5 mg day$^{-1}$ ($P=0.02$) and 10 mg day$^{-1}$ ($P=0.0078$). Percent changes in triglycerides of bonderline significance were observed ($P=0.05$). Also Bonferroni correction ($P<0.016$) affected significance, since percent changes of HDL-C after policosanol (5 mg day$^{-1}$) were not significantly different when compared with placebo group.

The effects of policosanol on copper-mediated lipid peroxidation parameters can be observed in Table 3. At baseline all groups showed similar values of the extent of LDL-C oxidation (lag phase and propagation rate). Policosanol 5 mg day$^{-1}$ ($P<0.01$) and 10 mg day$^{-1}$ ($P<0.001$) significantly and in dose-dependent manner increased lag phase from baseline, while in the placebo group it remained unchanged. The length of the lag phase after treatment with 10 mg day$^{-1}$ was significantly longer than that of placebo ($P<0.001$), whereas those obtained in the 5 mg group did not reach statistical significance compared with placebo. In addition, administration of policosanol (5 mg day$^{-1}$) decreased ($P=0.018$) the rate of conjugated diene generation when compared with placebo group. However, when Bonferroni adjustment was applied this level of significance was affected. Also, policosanol administered at 10 mg day$^{-1}$ significantly decreased the rate of conjugated dienes generation compared with baseline ($P<0.001$) and placebo ($P<0.01$).

Policosanol (5 and 10 mg day$^{-1}$) also dose-dependently reduced the susceptibility of LDL-C to macrophage-mediated oxidation as assessed by determination of TBARS generation (Table 4). Thus, mean TBARS accumulation decreased in the group treated with 5 mg day$^{-1}$ and 10 mg day$^{-1}$ from baseline ($P=0.027$ for 5 mg day$^{-1}$ and $P=0.012$ for 10 mg day$^{-1}$). However, after 8 weeks, TBARS levels in policosanol (5 mg day$^{-1}$) group did not decrease significantly using the Bonferroni correction ($P<0.016$). There was, however, a significant decrease in MDA generation after treatment with 5 and 10 mg day$^{-1}$ when compared with placebo ($P<0.01$).

Treatment was well tolerated. There were no significant differences in body weight, systolic and diastolic blood pressure, ALAT, ASAT and glucose levels. Thus, the present results suggest no drug-related disturbances. Five subjects withdrew from the study, none because of adverse experiences. Two subjects discontinued the study because they did not want to follow up and three because of protocol violation. Four subjects reported mild adverse experiences: two reported somnolence (one placebo and

**Table 4** Effects of policosanol treatment (5 and 10 mg day$^{-1}$) on the susceptibility of LDL-C to macrophage-mediated lipid peroxidation.

| | *TBARS (nmol MDA mg$^{-1}$ protein)* | |
|---|---|---|
| *Group* | *Baseline* | *8 weeks* |
| Placebo | $8.48 \pm 0.78$ | $9.15 \pm 0.66$ |
| Policosanol-5 | $8.55 \pm 0.29$ | $7.49 \pm 1.03$ ⋆++ |
| Policosanol-10 | $8.50 \pm 0.91$ | $5.76 \pm 1.01$ ⋆ [a]++ |

⋆ $P<0.05$. Comparison with baseline (Wilcoxon Test), [a] significantly different for the level of significance $P<0.016$ (Bonferroni ajustment). ++ $P<0.01$ Comparison with placebo (Mann Whitney *U*-test).

one policosanol, 5 mg), one reported asthenia (placebo) and another reported headache (policosanol, 5 mg).

## Discussion

The present study demonstrates that policosanol administered at doses (5 and 10 mg $day^{-1}$) within its conventional dosage as a lipid-lowering drug decreased the susceptibility of LDL-C to metal ion-dependent and macrophage-mediated lipid peroxidation. The methods selected for measuring LDL-C oxidation have shown to be valid, demonstrated by the reproducibility of the copper catalysed time-courses curves and the generation of MDA by macrophages at baseline and 8 weeks in the placebo group. Besides, baseline levels in lag time and propagation rate were similar than those previously described [5, 40], which also validates the method used in the present work to describe LDL-C oxidation.

Consistent with previous preclinical studies [31] policosanol (5 and 10 mg $day^{-1}$) significantly and in a dose-dependent manner increased the lag time and decreased maximum propagation rate. Of potential importance is the effect of policosanol treatment on LDL-C susceptibility to macrophage-mediated lipid peroxidation. Oxidative modification of LDL-C has been demonstrated to occur *in vivo*. Arterial wall cells, including macrophages, can mediate the lipid peroxidation of LDL-C [41]. Therefore, the finding that policosanol protected LDL-C against cell-mediated oxidation, suggest that policosanol might also protect LDL-C *in vivo*.

The effects of policosanol on lipid profile in the present study are expected and consistent with those observed in previous results in healthy volunteers [14]. Policosanol in a dose-dependent manner and significantly reduces the main efficacy variables of the lipid profile. Also, as previously described [15–29] policosanol was well tolerated.

Data from *in vivo* and *in vitro* studies suggest that the oxidation of LDL-C contributes critically to human atherosclerosis [5, 6]. Administration of antioxidants may protect LDL-C against oxidative modification and thus inhibit development of atherosclerosis disease. In this regard, evidence was shown the inhibition of LDL-C oxidation [40, 42, 43], and the regression of atherosclerosis [44, 45] by the antioxidant effect of probucol.

The antioxidant effects of other lipid-lowering drugs have been reported. Thus, some studies have shown that simvastatin [46] and fluvastatin [47] reduced the susceptibility of LDL-C to be oxidized *in vitro*. Moreover, a recent investigation has described that a metabolite of gemfibrozil inhibits LDL-C lipid peroxidation *in vitro* in a cell-free system [48]. However, oral administration of gemfibrozil (1200 mg $day^{-1}$) for 12 weeks to familial combined hyperlipidaemic patients, failed to protect LDL-C against copper ion-induced lipid peroxidation *in vitro* [49].

Therefore, since policosanol is a cholesterol-lowering agent, the combination of this property with its antioxidant effect might be of clinical importance. The reduction of both, plasma LDL-C concentration and LDL-C susceptibility to oxidation may be of importance in reducing atherosclerotic risk. The mechanism whereby policosanol prevents the susceptibility of LDL-C to lipid peroxidation is beyond the objective of this study. Nevertheless, taking into account the structure of the higher molecular weight aliphatic alcohols and their metabolism, effects of policosanol addressed to a change in the lipid structure of LDL-C cannot be ruled out.

Finally, since increased susceptibility to oxidation has been demonstrated in patients with hypercholesterolaemia [50–53] further studies are aimed to investigate the effect of oral administration of policosanol on the susceptibility of LDL-C isolated from hypercholesterolaemic patients to lipid peroxidation.

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