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Letter to the Editors

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In vivo oxidised cholesterol in atherosclerosis

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*Dear Editors,*

Evidence has been increasing the last several years that oxidative modification of LDL may play an important role in the pathogenesis of atherosclerosis [1,2]. Further support for the oxidative modification hypothesis is provided by the recent report of Salonen et al. [3] who found that the titre of autoantibodies to malondialdehyde-modified LDL was an independent predictor of the progression of carotid atherosclerosis. Although there is little doubt about the specificity of these antibodies to artificially oxidised LDL [4], direct evidence that LDL oxidation actually takes place *in vivo* is scarce; most research on oxidation of LDL involves oxidisability *in vitro*. We have measured circulating cholesterol oxidation products in WHHL rabbits. Six-month-old rabbits

(mean ( $\pm$  S.D.) serum cholesterol  $18.3 \pm 2.3$  mmol/l, mean serum triglycerides  $3.2 \pm 0.9$  mmol/l) were fed with either cholesterol-free control chow or chow mixed with the antioxidant probucol in a low dose (10 mg/kg per day). High doses of probucol are known to retard atherogenesis in these animals [5]. One control animal died during the study period. After 6 months of treatment blood was drawn into vacutainer tubes containing EDTA plus 10 mg of reduced glutathione, plasma was prepared immediately and 0.1 mg of the antioxidant butylated hydroxytoluene added per ml. This treatment effectively prevents oxidation *in vitro*: in plasma of 5 normal volunteers we found total oxysterol levels of  $1.36 \mu\text{mol/l}$  at time 0 and  $1.32 \mu\text{mol/l}$  after 6 month of storage at  $-80^\circ\text{C}$ . Cholesterol oxidation products were determined by a modification of our previously described method [6].

The mean concentration of serum cholesterol was unaffected in the control animals ( $18.6 \pm 1.3$

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TABLE 1

PLASMA LEVELS OF OXYSTEROLS ( $\mu\text{mol/l}$ ) IN 3 CONTROL AND 4 PROBUCOL-TREATED WHHL RABBITS (MEAN (S.D.))

	Control	Probucol	P
Total oxysterols	9.15 (1.33)	4.64 (0.58)	<0.01
7 $\alpha$ -Hydroxycholesterol	5.04 (0.33)	2.17 (0.64)	<0.001
5,6 $\alpha$ -Epoxycholestanol	0.78 (0.44)	0.39 (0.21)	NS
7 $\beta$ -Hydroxycholesterol	0.69 (0.23)	0.54 (0.09)	NS
5 $\alpha$ -Cholestanetriol	0.88 (0.10)	0.55 (0.09)	<0.01
7-Ketocholesterol	1.41 (0.39)	0.84 (0.11)	<0.01
25-Hydroxycholesterol	0.35 (0.13)	0.15 (0.02)	<0.05

vs.  $19.0 \pm 1.9$  mmol/l), whereas there was a slight decrease in the probucol treated animals ( $17.7 \pm 2.4$  vs.  $14.2 \pm 1.8$  mmol/l). Ingestion of a low dose of probucol caused a 50% difference in the levels of circulating cholesterol oxidation products (oxysterols) (Table 1).

This study provides direct evidence that oxidised lipoproteins occur in vivo in the circulation and that administration of low doses of probucol can prevent oxidation to a large extent, explaining its anti-atherogenic effect in WHHL rabbits.

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