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RESEARCH ARTICLE

Effects of Atorvastatin on LDL sub-fractions and peroxidation in type 1 diabetic patients: a randomised double-blind placebo-controlled study

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Abstract

Background Patients with diabetes have an increased risk of both developing and dying from cardiovascular disease, and, currently, more aggressive lipid-lowering targets are being recommended for these patients. Statins are widely and successfully used to correct dyslipidemia and prevent acute coronary episodes, but their effects on lipoprotein composition and peroxidation have not been fully investigated. We aimed to address this issue in type 1 diabetes mellitus.

Methods T1DM patients with atherogenic index (total/HDL-cholesterol > 4) were randomised double-blindly to group A ($n = 12$) that received Atorvastatin 40 mg/day and group P ($n = 12$) that received placebo. They were monitored for blood biochemistry, LDL sub-fractions and lipid peroxidation at inclusion, after 6 and after 12 weeks.

Results In group A, the 40% decrease in serum total and LDL cholesterol and 20% decrease in triglycerides was accompanied by a decrease in serum α-tocopherol from 46.4 ± 16.3 (mean $\pm$ SD) at inclusion to 32.2 ± 11.8 and 32.6 ± 14.0 μmol/L after 6 and 12 weeks respectively ($p < 0.001$ compared to group P by repeated-measures ANOVA). Relative to LDL + VLDL cholesterol, α-tocopherol increased by 40% ($p < 0.001$). Copper-induced LDL + VLDL peroxidation increased from 4891 ± 1325 at inclusion to 6821 ± 2291 and 7040 ± 1712 nmol TBARS/mg LDL + VLDL cholesterol produced in 3 h ($p = 0.004$). LDL sub-fractions shifted towards the less dense regions ($p = 0.03$).

Conclusions These results suggest that Atorvastatin lowers the antioxidant capacity of LDL and VLDL in T1DM. The mechanisms underlying these changes merit further investigation and should be taken into account when planning long-term primary prevention of CHD in diabetes.

Keywords Atorvastatin; lipid peroxidation; LDL sub-fractions; type 1 diabetes mellitus

Introduction

Diabetes mellitus (DM) is associated with a two- to fourfold increased risk of morbidity and mortality from atherosclerotic cardiovascular disease (CVD). This increased risk is only partly explained by the clustering of risk factors

such as hypertension, obesity and dyslipidemia in these patients [1,2]. Although high LDL (low-density lipoprotein) cholesterol, which is the major risk factor for CVD in the general population, is not more frequent in diabetes, other abnormalities of plasma lipid and lipoprotein metabolism are present and have been independently associated with the increased CVD risk in diabetes. However, these differ depending on the type of diabetes and on factors such as treatment, insulin resistance, obesity, physical exercise, diet and postprandial changes, microalbuminuria and family history. For example, whereas the dyslipidemia of type 2 diabetes (T2DM) and also of the insulin resistance syndrome is characterised by elevated very low density lipoproteins (VLDL), low HDL (high-density lipoprotein), increased apolipoprotein B and the presence of small, dense LDL [3] in type 1 (T1DM) dyslipidemia is mainly characterised by hypertriglyceridemia and low HDL cholesterol, which worsen during bad metabolic control and when nephropathy is present [4]. Moreover, the pathogenic consequences of the diabetes-induced dyslipidemia are further worsened by several pathologic effects of hyperglycemia such as abnormalities in cellular function [5,6], glycation [7] and oxidative stress [8]. These all lead to increased susceptibility to lipid peroxidation [9] and to the accumulation of products of oxidative damage to lipids [10], proteins [11] and DNA [12].

Thus, although elevated LDL cholesterol remains the indispensable condition for the initiation and progression of atherosclerosis in both diabetic and non-diabetic subjects [13], all these other diabetes-related abnormalities exaggerate the risk of even moderately elevated LDL cholesterol [14,15].

These considerations imply that the goals of LDL cholesterol–lowering treatment should be more strict in diabetes [15,16]. Several clinical trials have confirmed that aggressive LDL-lowering therapy with statins reduced recurrent coronary heart disease CHD events in diabetic patients [17,18], even in those with low LDL cholesterol [19].

Although most of these data stem from studies conducted in type 2 DM patients and less information is available on the effects of statins in type 1 DM [20], the same guidelines are currently applied to type 1 DM patients, especially if other risk factors such as nephropathy and hypertension are present [15].

However, there is still insufficient information on the effects of these cholesterol-lowering drugs on other pathogenic events involved in the atheroma-plaque formation. It is not totally known how the properties and composition of lipoproteins are affected and how these can affect lipid peroxidation, foam cell accumulation and eventually atheroma-plaque formation in T1DM.

We proposed to investigate this question by studying the effects of Atorvastatin, a currently used, more potent statin [21], on lipoprotein composition, LDL sub-fractions and peroxidation in T1 diabetic patients with high serum levels of cholesterol.

Research design and methods

Patient population and study design

Type 1 diabetic patients attending the polyclinic at the Antwerp University Hospital were screened according to their blood lipids. Type 1 diabetes was established by a history of insulin-dependency and fasting plasma C-peptide levels <0.2 nmol/L. Those with an atherogenic index (total cholesterol/HDL cholesterol) ≥ 4 were randomly assigned to 2 groups in a double-blind manner. Exclusion criteria were intake of multivitamin preparations and drugs that interfere with the oxidant(–)antioxidant status. Group P ($n = 12$) received placebo and group A ($n = 12$) received 40-mg Atorvastatin® daily. Patients were monitored 3 times: at inclusion, and after 6 and 12 weeks of treatment with medication or placebo.

The experimental protocol was in accord with the Helsinki declaration and was approved by the ethical commission of the University Hospital. Participating subjects signed an approved consent form.

All patients were on a standard diet for diabetes consisting of 7.5 to 8.5 MJ/day (50% of the energy as carbohydrates, 20% as protein and 30% as fats). This diet assures a daily intake of at least 3-mg vitamin E, 3000-μg vitamin A, 150-mg vitamin C and 26-mg flavonoids.

Analytic methods

Fasting blood samples were collected at each visit.

Routine blood tests (blood count, urea nitrogen, creatinine, uric acid, glucose, liver enzymes, protein, albumin, total and HDL cholesterol, triglycerides, sodium, potassium, calcium, parathyroid hormone, iron and TIBC, bilirubin, C-reactive protein, alkaline phosphatase, fibrinogen, haematocrit, haemoglobin, blood formula) were analysed in the routine laboratory of the clinic. Total analytical variability, expressed as coefficient of variation (CV, was 2%, 1.9% and 0.9% for total cholesterol, HDL cholesterol and triglycerides respectively. LDL cholesterol was calculated according to the Friedewald equation [22]. ApoA1 and B were measured by turbidimetry (Turbitimer, Dade Behring, Marburg GmbH, Germany). The ratio LDL cholesterol/apoB to indicate the presence of small, dense LDL particles was calculated according to the formula of Hattori [23]. Glycated haemoglobin (HbA_{1c}) was measured using a high-performance liquid chromatography (HPLC) cation-exchange column (Modular Diabetic monitoring system BIO-RAD, California, USA), CV was 1.5%. *Oxidant(–)antioxidant balance* was evaluated by measuring the concentrations of individual antioxidants and the susceptibility of lipoproteins to oxidative attack *in vitro* (peroxidability). Vitamins E and A in serum were measured by HPLC (Shimadzu, Bio-Rad reverse-phase C18 with a 100% methanol mobile phase) with detection at 292 and 325 nm respectively and a CV of 10 and 13% respectively [24]. The susceptibility of LDL and

VLDL to copper-catalysed oxidation was measured by isolating these two groups of lipoproteins (non-HDL) by dextran sulphate/$MgCl_2$ precipitation and incubation of a suspension containing 200 μg/mL of cholesterol with 46 μM $CuSO_4$ for up to 180 min at 37 °C, during which aliquots were taken every 30 min for the measurement of thiobarbituric acid reactive substances (TBARS). Fluorescence at 360-nm excitation and 440-nm emission was monitored continuously. Three phases were measured: (1) the lag time (expressed in minutes), during which fluorescence does not increase significantly; it quantifies the capacity of the antioxidants within the lipoproteins to retard the initiation of oxidation; (2) the slope of the propagation phase, during which fluorescence increases rapidly and which indicates the velocity of oxidative changes in the apoB and (3) the saturation phase, during which fluorescence reaches a plateau and which gives an estimate of the total amount of lipid oxidised. CVs of these parameters ranged from 3 to 11% [25].

LDL sub-fractions were measured in EDTA plasma using the lipoprint LDL system, on the basis of polyacrylamide gel electrophoresis (Lipoprint LDL Kit, Quantimetrix, CA, USA). Up to seven LDL sub-fractions can be separated as well as one VLDL band, 3 midbands and one HDL band. When the VLDL position is taken as 0.00 and HDL as 1.00, the 'large LDL' bands have the relative positions 0.30 and 0.36; 'small LDL' bands are at the positions 0.41, 0.46, 0.51, 0.56 and 0.61. In the patient samples, the presence or absence of each band was noted by comparison with a template. In order to quantify the sub-fractions, the optical density of each LDL sub-fraction was scanned (Appraise densitometer, Beckman Instruments, Inc. Diagnostic System Group, Brea CA) and expressed as a percent of the total LDL (sum of all the fractions).

Homocystein was measured by HPLC (Waters Nova-Pak C18 with 0.1 M sodium acetate pH 4.1 as mobile phase) and fluorescence detection at 384-/514-nm excitation/emission [26].

Statistical methods

Data were analysed using SPSS software (version 10, Chicago, IL). Results were expressed as means ± SD, and two-tailed p-values < 0.05 were considered significant. Between-group comparisons at inclusion were done by t-test or Mann(−)Whitney test for the non-Gaussian variables. The effect of medication versus placebo during the course of the three visits was analysed by repeated Analysis of Variance measures (ANOVA). Data was analysed for the changeover time during the course of the various visits (within-group comparison) and for differences between the two groups as regards these time-related changes, that is, for the interaction between medication and time (between-group comparison of the change over time). In view of the significant between-group differences for some lipid parameters at inclusion, the RM ANOVA also included the analysis of the contrasts between inclusion values and those after 6 and 12 weeks. These contrasts were also compared between the two groups.

Table 1. Patient characteristics at inclusion

Characteristic	Group A ($n = 12$)	Group P ($n = 12$)
Age (years)[a]	44 (25–66)	44 (20–74)
Sex (male/female)[b]	9/3	9/3
Duration of diabetes (years)[a]	18 (15–37)	15 (3–37)
Fasting glycemia (mmol/L)	8.39 ± 1.17	9.06 ± 2.44
HbA_{1c} (%)	7.80 ± 1.18	8.02 ± 1.05
Insulin dose (U/day)	51 ± 18	57 ± 14
U/kg body weight	0.65 ± 0.17	0.73 ± 0.18
BMI (kg/m^2)	25.8 ± 2.7	27.1 ± 3.1
Blood pressure systolic (mm Hg)[a]	128 (110–140)	120 (105–180)
Blood pressure diastolic (mm Hg)[a]	78 (65–85)	80 (70–92)
Nephropathy (no/yes)[b]	12/0	11/1
Retinopathy (no/yes)[b]	5/7	6/6
Neuropathy (no/yes)[b]	5/7	6/6
Peripheral arteriopathy (no/yes)[b]	11/1	9/3
Coronary heart disease (no/yes)[b]	7/5	8/4

Values are expressed as means ± SD, [a] as median (minimum-maximum) for non-Gaussian distributed data or [b] as number of observations. There were no significant differences between the two groups at inclusion.

Results

Randomization at inclusion resulted in two comparable groups of patients as regards clinical characteristics, complication profile and routine biochemistry (Table 1). Only one patient in group P had intermittent microalbuminuria (<20 μg/min) and about half of all patients had some degree of chronic complication. When comparing serum lipids at inclusion, group A had significantly higher levels of total, LDL cholesterol and apoB but comparable atherogenic index (Table 2). In this group, these parameters decreased by 40–50% after 6 weeks and no further after 12 weeks Atorvastatin. They did not change in group P ($p < 0.0001$ for the within- and between-groups comparisons after correcting for the significant difference at inclusion). Serum triglycerides decreased by 20% (n.s. when contrasting the change from inclusion to 6 weeks and $p < 0.04$ from inclusion to 12 weeks). The ratio LDL cholesterol/LDLapoB taken as a measure of the presence of small, dense LDL particles decreased slightly in group A (n.s.) and increased slightly in group P (n.s.) ($p = 0.015$ for the comparison between the two groups).

Clinical and biochemical parameters such as body weight, insulin dose, HbA_{1c}, glycemia, serum proteins and transaminases did not change in either group. Serum ureum increased slightly ($p = 0.04$) in both groups and creatine phosphokinase increased in group A from 118 ± 64 U/L at inclusion to 137 ± 69 after 6 weeks ($p = 0.025$ when compared to group P) and to 126 ± 44 after 12 weeks (n.s.). Diastolic blood pressure decreased in both groups ($p = 0.054$) to the same extent ($p = 0.18$ when comparing the evolution in the two groups).

Analysis of plasma at inclusion with Lipoprint showed that only 6 out of the 24 patients had peaks at position

Table 2. Serum lipids in type 1 diabetic patients before and after receiving Atorvastatin 40 mg/day or placebo

Variable	Group A ($n = 12$) At inclusion	Group A After 6 weeks Atorvastatin	Group A After 12 weeks Atorvastatin	Group P ($n = 12$) At inclusion	Group P After 6 weeks Placebo	Group P After 12 weeks Placebo	p-value
Total cholesterol (mmol/L)	6.80 ± 1.66	4.03 ± 0.80	4.19 ± 1.11*	5.56 ± 1.01§	5.79 ± 0.88	6.05 ± 1.22	<0.001
LDL cholesterol (mmol/L)	4.81 ± 1.60	2.25 ± 0.62	2.30 ± 0.88*	3.60 ± 0.93§	3.75 ± 1.01	4.01 ± 1.06	<0.001
HDL cholesterol (mmol/L)	1.37 ± 0.29	1.37 ± 0.36	1.37 ± 0.31	1.32 ± 0.34	1.27 ± 0.31	1.35 ± 0.29	n.s.
Atherogenic index (total/HDL)	5.10 ± 1.65	3.04 ± 0.75	3.16 ± 1.08*	4.36 ± 0.92	4.74 ± 0.95	4.53 ± 0.55	<0.001
Triglycerides (mmol/L)	1.33 ± 0.31	0.89 ± 0.32	1.10 ± 0.76	1.42 ± 0.46	1.29 ± 0.45	1.40 ± 0.73	0.04
Apoprotein A1 (mg/dL)	122 ± 25	109 ± 24	111 ± 21	116 ± 24	108 ± 18	112 ± 24	n.s.
Apoprotein B (mg/dL)	142 ± 37	75 ± 18	82 ± 24*	114 ± 20**	117 ± 16	119 ± 24	<0.001
Ratio ApoA1/ApoB	0.89 ± 0.20	1.50 ± 0.33	1.45 ± 0.38*	1.03 ± 0.25	0.96 ± 0.22	0.95 ± 0.15	<0.001
Ratio LDL cholesterol/LDLapoB[b]	1.53 ± 0.12	1.49 ± 0.26	1.37 ± 0.27	1.46 ± 0.22	1.59 ± 0.21	1.63 ± 0.30	0.015
Lipoprotein (a) (mg/dL)[a]	13.7 (<10–97)	17.5 (<10–119)	15.1 (<10–113)	10 (<10–87)	10 (<10–94)	10 (<10–90)	n.s.

Values are expressed as means ± SD, [a] as median (minimum–maximum) for non-Gaussian distributed data. Data was analysed by repeated-measures ANOVA. The p-values denote the between-group comparison of the change over time, when contrasted to visit 1. The ratio LDL cholesterol/apoB was calculated according to the formula of Hattori [23]. * Denotes $p < 0.0001$ for the overall change over time (during the course of the three visits) within the group. ** Denotes $p < 0.05$ for the between-group comparison at inclusion.

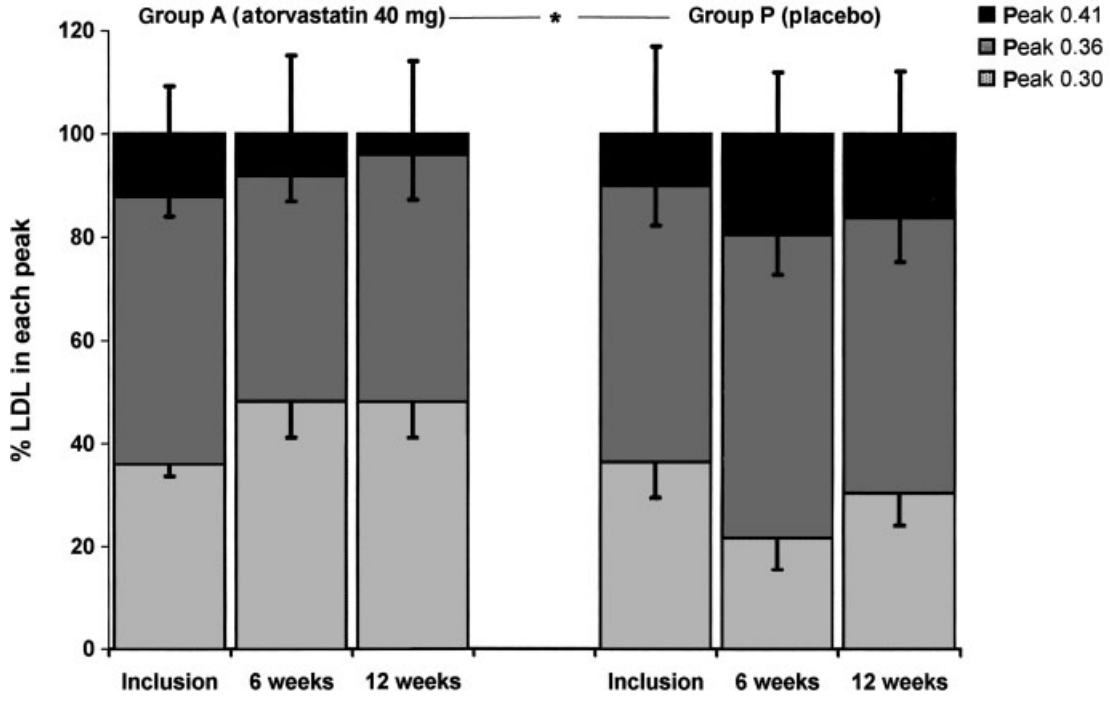

Figure 1. LDL sub-fractions at inclusion, after 6 and after 12 weeks in group A (receiving Atorvastatin 40 mg/day) and in group P (receiving placebo). Shown are mean ± SEM of the percentage of total LDL present in the peak at position 0.30 (light grey), at position 0.36 (dark grey) and at position 0.41 (black). *denotes $p = 0.03$ for the between-group comparison of the change over time

0.41, which is considered to be 'small LDL'. These patients tended to have higher serum triglycerides (1.603 ± 0.348 vs 1.286 ± 0.383 mmol/L, $p = 0.09$) and lower LDL-cholesterol/apoB ratio (1.40 ± 0.24 vs 1.54 ± 0.14, $p = 0.11$) but did not differ as regards cholesterol levels, body mass index (BMI) or insulin dose. Most patients had peaks at positions 0.30 (21 patients) and at 0.36 (22 patients), which are considered to be 'large LDL'.

As seen in Figure 1, in group A there was a shift of LDL sub-fractions towards the VLDL region with a 20% increase in the proportion of LDL in the 0.30 peak and a corresponding decrease in the 0.36 peak after Atorvastatin. The pattern of change in group P was in the opposite direction ($p = 0.03$ for the between-group difference in the change over time). After 6 weeks, this shift towards the less denser LDL positions had occurred in 11 patients out of group A in contrast to only 4 from group P ($\chi^2 7.8$, $p = 0.020$). Between 6 and 12 weeks, there were no further significant changes in LDL sub-fraction distribution. Of the 4 patients with a 0.41 peak in group A, 2 still had this peak after 6 weeks and only 1 after 12 weeks.

Lipoprotein peroxidation *in vitro* and serum vitamin E, A and homocystein levels were comparable in the two groups at inclusion (Table 3). Serum vitamin E levels were strongly related to LDL cholesterol ($r = 0.60$, $p < 0.0005$ at inclusion, $r = 0.49$, $p = 0.016$ after 6 weeks and $r = 0.60$, $p = 0.002$ after 12 weeks) but

not to HDL cholesterol or triglycerides. In a multiple regression model including all lipid parameters at inclusion, total cholesterol explained 34% and uric acid a further 15% of the variance of serum vitamin E. At inclusion, there were no significant correlations between serum vitamin E and lipid peroxidation parameters. As regards lipids, only HDL cholesterol was related to TBARS production after incubation with copper for 120 to 180 min ($r = -0.60$, $p = 0.002$ after 180 min).

In contrast to group P, in which no significant changes were detectable in the course of the 12 weeks of the study, in group A serum vitamin E decreased by 30% ($p = 0.024$ for the within-group comparison and $p < 0.001$ for the comparison with group P). However, when expressed relative to total lipids or to LDL + VLDL cholesterol, it increased by respectively 12% ($p = 0.04$ but n.s. when compared to placebo) and 40% ($p < 0.001$). There were no further changes between 6 and 12 weeks.

In vitro peroxidation of LDL + VLDL (total TBARS) increased by 40% ($p = 0.004$) and the velocity of formation of fluorescent peroxidation products tended to increase ($p = 0.057$ for the overall change and $p = 0.003$ when contrasting the value at 12 weeks to that at inclusion). As seen in Figure 2, the increase in copper-induced lipid peroxidation was significant at all time points ($p < 0.01$ for the overall within-group comparison) and increased further from 6 to 12 weeks' Atorvastatin.

Discussion

Atorvastatin belongs to the statin group of cholesterol-lowering drugs that inhibit HMG CoA-reductase. It is said to have a longer duration of action [27] and to specifically target the liver, resulting primarily in a decreased synthesis of VLDL particles [28]. This drug has been indicated for patients with primary hypercholesterolemia, homozygous familial hypercholesterolemia and mixed dyslipidemia [29], and more recently, trials have and are being conducted in T2DM patients [30,31]. In these patients, doses of 10 to 80 mg/day lead to decreases in serum concentrations of total and LDL cholesterol (by 35–61%), apoprotein B100 (by 35–50%) and triglycerides (by 14–45%) [21]. In our study, 40-mg Atorvastatin daily given for 6 and 12 weeks to T1DM patients with an elevated atherogenic index also lowered LDL cholesterol and apoprotein B100 by 40 to 50% together with a slight decrease in triglycerides (by up to 20%). In one of the few Atorvastatin studies conducted in T1DM patients, similar decreases in LDL cholesterol (by 48%) were accompanied by an improvement in endothelial function [32]. Whereas in T2DM, Atorvastatin treatment also caused significant increases in HDL cholesterol (by 6–26%) together with improvements in atherogenic indexes such as microalbuminuria, diastolic blood pressure and fibrinogen [30,33,34], no such changes were seen in our group of T1DM patients. Neither was there any significant change in parameters of metabolic control such as glycated haemoglobin, as also seen by Tanaka in T2DM [35]. In other studies, improvements in glycated haemoglobin, insulin resistance and respiratory quotiënt were directly related to the improvement in serum triglycerides [36].

Again in contrast to T2DM [37], the presence of small, dense LDL is not frequent in T1DM unless nephropathy is present [38] or, as observed in the DCCT trial, when excessive weight gain occurs as a result of intensive insulin therapy [39]. Nevertheless, it has been shown that even in non-diabetic subjects, LDL size is negatively correlated with its glycation [40] and that glycated LDL from T1DM patients has structural and fluidity abnormalities [41], is richer in triglycerides and in general behaves more atherogenically [42]. Using the Lipoprint method, small

Table 3. Serum vitamins and lipoprotein peroxidation in type 1 diabetic patients before and after receiving Atorvastatin 40 mg/day or placebo

	Group A ($n = 12$)			Group P ($n = 12$)			
Variable	At inclusion	After 6 weeks Atorvastatin	After 12 weeks Atorvastatin	At inclusion	After 6 weeks Placebo	After 12 weeks Placebo	*p*-value
Serum α-tocopherol (μmol/L)	46.4 ± 16.3	32.2 ± 11.8	32.6 ± 14.0*	41.5 ± 15.5	44.0 ± 12.9	44.7 ± 15.7	<0.001
(μmol/mmol total lipid)	5.68 ± 1.36	6.48 ± 1.64	6.19 ± 1.84*	5.95 ± 1.78	6.05 ± 1.97	6.03 ± 1.64	n.s.
(μmol/mmol non-HDLcholesterol)	8.57 ± 2.21	12.05 ± 3.35	11.80 ± 3.76**	9.90 ± 2.93	9.85 ± 2.97	9.52 ± 2.48	<0.001
Serum retinol (μmol/L)	3.00 ± 0.72	2.94 ± 0.86	3.18 ± 0.82	3.06 ± 1.11	3.14 ± 0.93	3.35 ± 1.10	n.s.
Serum homocystein (μmol/L)	9.85 ± 3.14	9.87 ± 2.93	10.02 ± 4.64	9.00 ± 2.02	9.09 ± 2.60	8.49 ± 2.26	n.s.
In vitro lipid peroxidation[a]							
Total TBARS production	4891 ± 1325	6821 ± 2291	7040 ± 1712*	5685 ± 1226	5712 ± 1895	5533 ± 1873	0.004
Fluorescence lag time (min)	104 ± 19	97 ± 24	100 ± 21	109 ± 26	98 ± 18	104 ± 29	n.s.
Fluorescence slope (FU/min)	0.38 ± 0.08	0.42 ± 0.14	0.58 ± 0.15	0.43 ± 0.13	0.47 ± 0.28	0.41 ± 0.10	0.057

Values are expressed as means ± SD. Data was analysed by repeated-measures ANOVA and * denotes $p < 0.05$ and ** $p < 0.001$ for the overall change over time (during the course of the three visits) within the group. The *p*-values denote the between-groups comparison of the changes over time.
[a]*In vitro* lipid peroxidation of LDL ± VLDL (non-HDL) was determined by incubating with copper and measuring the concentration of TBARS every 30 min for a total of 3 h.
Total TBARS production was calculated as area under the curve of the time course (see Figure 2) and expressed as nmol MDA equivalents/mg non-HDL cholesterol × minutes. The formation of fluorescent products was monitored continuously at 360/440 nm excitation/emmission to measure lagtime and velocity of formation (slope).

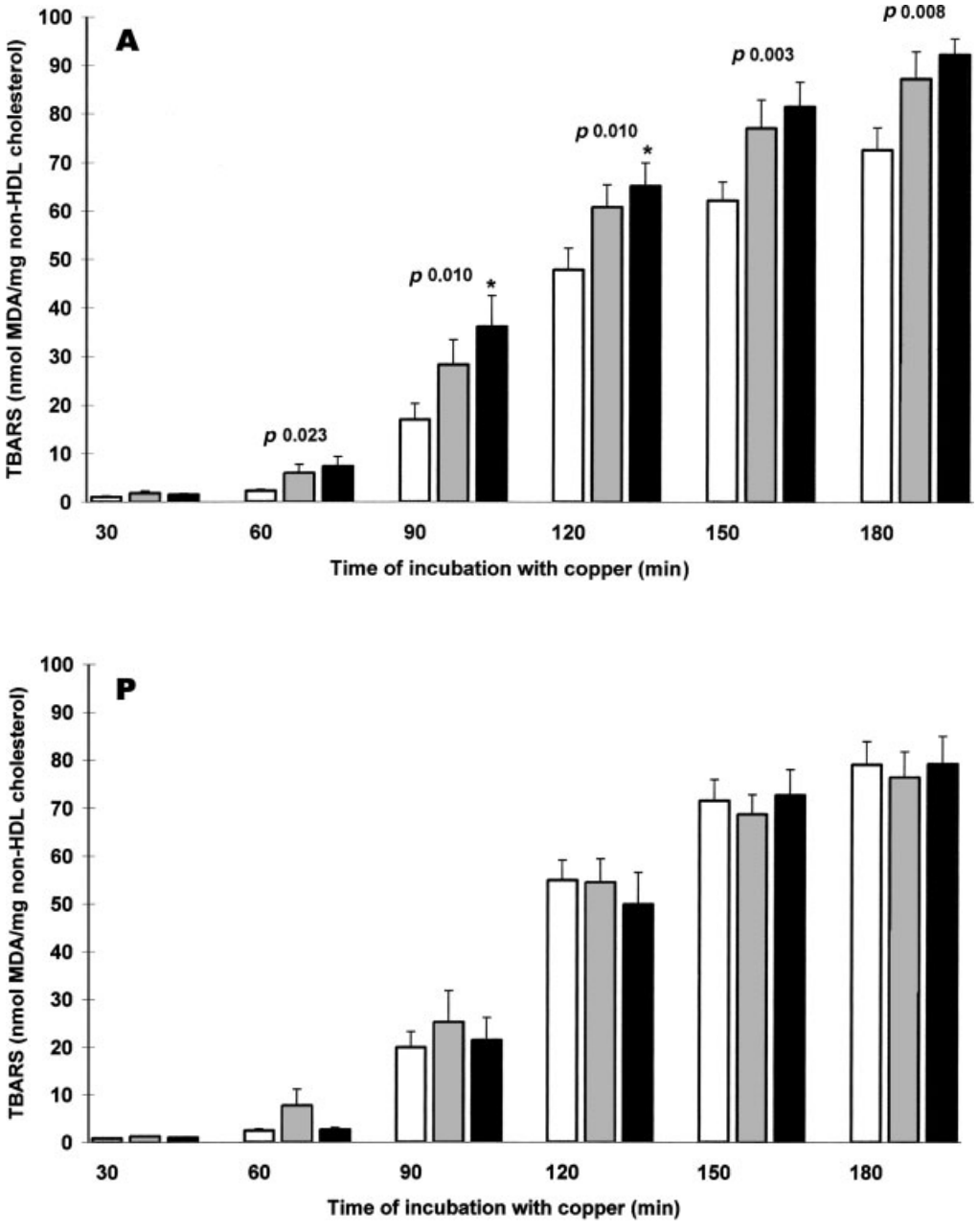

Figure 2. Time course of the production of thiobarbituric-reactive substances (TBARS) during *in vitro* incubation of LDL and VLDL with copper in (A) the group receiving Atorvastatin 40-mg daily and (P) in the group receiving placebo. Shown are mean + SEM, white bars at inclusion, light grey after 6 weeks and black after 12 weeks. The p-value shown denotes the overall change in time within the group. *denotes $p = 0.01$ for the change between 6 and 12 weeks

LDL peaks corresponding to a diameter <25.5 nm are found in positions 0.41–0.61 [43]. In our group of patients, with no nephropathy or excessive BMI, none had peaks in regions 0.46–0.61 and only 6 out of 24 patients had LDL peaks in position 0.41, the largest of the 'small LDL'. This small group of patients tended to have higher triglycerides, as also seen in T2DM with small, dense LDL [37] and a lower LDL-cholesterol/LDLapoB ratio. However, this ratio was still well above the threshold value of 1.20. Values under this threshold reflect preponderance of small, dense LDL, as demonstrated by Hattori in various macroangiopathies with dyslipidemia [23]. The Atorvastatin-induced decreases in cholesterol and triglyceride levels were accompanied by changes in lipoprotein density as illustrated by the shift of LDL sub-fractions towards the VLDL region with relative enrichment in the 'large LDL' and the almost total disappearance of the 'small LDL' peaks. In T2DM, there is a marked increase in mean LDL particle diameter from the small, dense (25.3 nm) towards the intermediate subclass (26.5 nm) [44]. However, the shift from the intermediate towards the large LDL in our group of T1 patients was not accompanied by an increase in the cholesterol/apoB ratio in these particles, as would be expected from Hattori's calculations. It should be stressed here that the ratio and the LDL density distribution in this group of T1DM

patients was near-normal, thus making it more difficult to demonstrate the same changes as in patients with more outspoken dyslipidemias. It is plausible that the effects of Atorvastatin would have been more clear-cut if given to T1 patients having a more pathological and denser LDL profile (e.g. patients with nephropathy, high BMI or insulin resistance). Further investigations should also be conducted on the influence of factors other than cholesterol/apoB (e.g. other lipids) on particle density and on the possibility that these might exert a different influence in the normal and pathological ranges.

It is also interesting to note that the response to Atorvastatin differs in the various types of dyslipidemias. Whereas in T2DM the decrease in cholesterol was uniform in all LDL sub-fractions, in non-diabetic subjects with hypercholesterolemia the Atorvastatin-induced decrease in cholesterol was more pronounced in the less dense sub-fractions [34,45]. In combined hyperlipidemia, cholesterol reduction was preponderant in the dense LDL and was related to the decrease in VLDL1 levels, which in turn were associated with a reduction in cholesteryl ester transfer protein CETP activity [46]. Even though our T1DM patients had no outspoken hypertriglyceridemia and the Atorvastatin-induced decrease in triglycerides was modest, the changes in density that we observed might also be attributed to a decrease in exchanges between lipoproteins (cholesterol ester transfer in between HDL, LDL and VLDL). This would lead to a decrease in triglyceride-rich particles, which are the precursors of the small, dense LDL. In this context, it is difficult to assess the possible interference by exogenous insulin and by the degree of insulin resistance.

In this group of patients, serum α-tocopherol at inclusion was high (about 45 μmol/L) when compared to values found in normolipidemic T1DM patients by our group in the same period of time (30 μmol/L) [47]. This is probably explained by the hypercholesterolemia in these patients, as suggested by the strong correlation between serum α-tocopherol and cholesterol. Atorvastatin induced a 30% decrease in α-tocopherol when expressed in μmol per litre serum, but a 40% increase when expressed per mmol LDL and VLDL cholesterol. In normocholesterolemic T2DM, Atorvastatin also induced decreases in plasma vitamin E, but the ratio to LDL cholesterol was maintained [48]. In hypercholesterolemic patients, there was a 22% increase relative to LDL after 3 years of pravastatin [49].

As already observed by us and by other groups, copper-induced production of TBARS and fluorescent products is higher in T1DM than in non-diabetic subjects [25]. In unsupplemented subjects, it is not related to serum levels of α-tocopherol [50,51]. Only HDL cholesterol correlated negatively with peroxidation in this group of patients. After Atorvastatin, there was a 40% increase in copper-induced production of TBARS. This change is paradoxical considering the maintenance of HDL levels and the increase in LDL α-tocopherol relative to cholesterol. It would also be expected that the shift of LDL towards the less dense sub-fractions would be accompanied by a decrease in peroxidation. It is well documented that small, dense LDL is more prone to peroxidation [52] and that in T2DM the lag time correlates positively with the LDL size [53], the threshold for LDL oxidability being set at diameters smaller than 25.5 nm [43]. The Atorvastatin-induced increase in LDL and VLDL peroxidation that we observed in T1DM contrasts with decreases observed in hypercholesterolemic patients after longer treatments with other statins [49,54]. We do not know if this difference is intrinsic to T1DM or is due to differences in the methods used to evaluate peroxidation or in the structure of the various statins. For example, Lovastatin has antioxidant properties related to the presence of a lactone ring that is capable of accepting electrons from free radicals easily [54].

Changes in other factors affecting the susceptibility of lipoproteins of T1DM subjects to peroxidation should also be considered. For example, the unequal effects of Atorvastatin on serum cholesterol, phospholipids and triglycerides, as seen after pravastatin [49], and the changes in LDL particle density distribution can be accompanied by changes in LDL components such as fatty acids, protein and antioxidants. If the polyunsaturated fatty acid PUFA content increases, changes in fluidity enhancing the migration of free radicals through the particle and increased presence of pre-formed lipid hydroperoxides might render the lipoprotein particle more susceptible to copper-induced peroxidation [55,56].

And lastly, there might also be changes in the concentration of other antioxidants that, although present in lower concentrations, are actively involved in the recycling of the tocopheryl radical within the LDL particle. For example, coenzyme Q10 synthesis is also dependent on HMG CoA-reductase activity [57]. It has indeed been shown that other statins lead to a 20 to 30% decrease in serum coQ10 [58]. In healthy volunteers, doses of 10-mg Atorvastatin/day for 4 weeks did not decrease coQ10 serum levels [59]. Further studies are needed to investigate the consequences of higher doses in both types of diabetic patients. If, as postulated by some authors, coQ10 is more effective than α-tocopherol in increasing the resistance of LDL to the initiation of oxidation in conditions that simulate the *in vivo* situation [60], possible statin-induced depletion of coQ10 in the LDL particle might in the long-run counteract some of the anti-atherogenic benefits derived from cholesterol-lowering.

It should be stressed that *in vitro* methods to measure lipoprotein peroxidability only measure one or two of the steps in the peroxidation cascade and therefore do not wholly reflect LDL particle atherogenicity. Moreover, these methods do not give an insight into other factors surrounding the lipoprotein particle, which also play an important role in lipid peroxidation *in vivo*. Nevertheless, the increase in *in vitro* peroxidation induced by Atorvastatin merits further investigation and should be taken into account when planning the primary prevention of the dyslipidemia in diabetes. Indeed, lipid peroxidation plays its fundamental role in the early stages

of atheroma-plaque formation and this may take place many years before the clinical episodes.

The efficacy of statins in preventing clinical events of coronary heart disease has unequivocally been shown in the 4S, CARE (cholesterol and recurrent events) and HPS (heart protection study) trials [17–19]. However, these were secondary prevention trials, the diabetic patients were predominantly T2DM and the beneficial effects were attributed not only to the lowering of serum cholesterol but also to other pleiotropic effects of statins [61,62]. The long-term effects of statins given in primary prevention will only be available after the CARDS (Collaborative Atorvastatin in diabetes study), DALI (Diabetes Atorvastatin lipid intervention study) and ASPEN (Atorvastatin study for the prevention of coronary heart disease in MIDDM) trials are completed and provided they include a sufficient number of T1DM patients [31]).

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