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Low-density lipoprotein (LDL) behavior after in vitro oxidation in three groups of diabetics☆

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Abstract

Diabetes is associated with increased morbidity and mortality resulting from cardiovascular disease. It has been established that oxidized LDLs are involved in the genesis of atherosclerosis. We have studied LDL oxidizability in three types of diabetics: insulin-dependent diabetes mellitus (IDDM), non-insulin-dependent diabetes mellitus (NIDDM) and insulin-treated diabetes mellitus type 2 (ITDM2) and a control group. LDLs have been isolated using ultracentrifugation and oxidized by addition of cupric chloride. With the oxidation kinetic, we calculated the lag time and the oxidation rate. Total fatty acids, α-tocopherol, and malondialdehyde (MDA) have been measured in native and oxidized LDLs.

Diabetics have a significantly lower lag time and a lower level of α-tocopherol. Oxidized LDLs of diabetics show an important decrease of their polyunsaturated fatty acids with an increase of MDA compared to the control. Our study demonstrates a higher susceptibility to oxidation of LDL from diabetics; this can be explained by alteration in LDL composition or by the oxidative process occurring in this disease. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Low-density lipoprotein; In vitro copper oxidation; Diabetes

1. Introduction

Macrovascular disease represents a major cause of morbidity and mortality in diabetics [1]. Low-density lipoproteins (LDLs) are involved in the pathogenesis of atherosclerotic lesions [2], through modifying processes such as oxidation. Modifications of LDL prevent their catabolism via the LDL receptor pathway. Oxidized LDLs are taken up by the non-down-regulated scavenger receptor of macrophages, leading to the formation of foam cells [3,4]. We have studied in vitro susceptibility to the oxidation of LDL isolated from the plasma of three groups of diabetics and measured the total fatty acids, malondialdehyde (MDA), and α-tocopherol in native and oxidized LDL.

2. Subjects and methods

2.1. Subjects

Blood samples were obtained under fasting conditions from 100 patients with diabetes mellitus. Some NIDDM patients, after about 10 years, become resistant to oral medication and then receive insulin treatment. They represent another group of diabetics called ITDM2. Anthropometric and clinical data of diabetics and controls are summarized in Table 1.

2.2. LDL isolation

Blood was collected into Vacutainer® tubes containing sodium edetic acid (EDTA). LDL ($d = 1.09–1.063$ kg) was isolated by ultracentrifugation (Beckman TL 100, France) in KBr gradient according to Esterbauer et al. [5].

2.3. LDL oxidation

After an extensive dialysis of LDL against sodium phosphate buffer (0.01 M, pH 7.4), in darkness for 24 h

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Table 1
Anthropometric and clinical data of diabetics and controls

	Controls	IDDM	NIDDM	ITDM2
n	12	18	55	27
Age (years)	38.6 ± 15.5	50.4 ± 17.6	59.4 ± 11.6 *	68.74 ± 12.4 *
BMI (kg/m^2)	22 ± 2	24.92 ± 4.03 *	29.61 ± 5.42 *	27.84 ± 4.53 *
Disease duration (years)		18.64 ± 12.69	12.41 ± 9.18	13.45 ± 8.52 NIDDM 5.84 ± 4.60 ITDM2
HbA1c (%)	5 ± 0.9	8.7 ± 1.5 *	10.2 ± 2.2 *	9 ± 2.3 *
Triglycerides (mM/l)	0.75 ± 0.31	1.30 ± 0.55 *	2.20 ± 2.05 *	1.54 ± 0.94 *
HDL-cholesterol (mM/l)	2.17 ± 0.41	1.66 ± 0.45 *	1.20 ± 0.41 *	1.27 ± 0.37 *
LDL-cholesterol (mM/l)	3.10 ± 0.95	3.40 ± 0.86	3.18 ± 1.04	3.26 ± 0.83

* $P < 0.05$ diabetics versus controls.

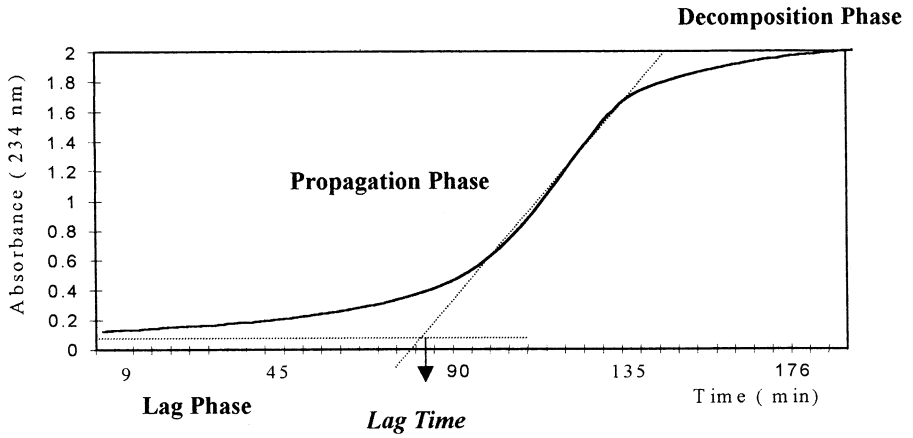

Fig. 1. Kinetics of conjugated diene formation by continuous monitoring of absorbance at 234 nm.

(three baths), we proceed to copper-induced oxidation. $CuCl_2$ was added to LDL diluted in dialysis buffer. The kinetics of the LDL oxidation was monitored by the continuous change in absorbance of conjugated dienes at 234 nm at 37°C for 3 h. The absorbance curve is divided into three phases: lag phase, propagation phase, and decomposition phase.

2.4. Plasma lipids (mM/l)

HDL-cholesterol, LDL cholesterol, and triglycerides were measured with enzymatic methods.

2.5. Glycated hemoglobin (HbA1c) (%)

Glycated hemoglobin was separated on an analytical column (4 mm i.d. × 15 cm) using a BioRad Diamat™ fully automated glycated hemoglobin analyzer system with ion-exchange high-performance liquid chromatography (HPLC) principles.

2.6. Plasma total fatty acids (mM)

The total fatty acid composition of plasma was evaluated by capillary gas liquid chromatography (GLC). The concentration of each fatty acid was expressed in absolute value (mg/l) by adding a known quantity of 17:0 fatty acid in the sample as internal standard.

Briefly, GLC conditions were a fused silica capillary column SP-2380 (SUPELCO); temperature programmed from 120 to 259°C; detector at 280°C; injector at 270°C. The carrier gas was helium.

Table 2
Lag time, MDA and α-tocopherol of LDL in diabetics and controls

	Controls	IDDM	NIDDM	ITDM2
Lag time (min)	66.25 ± 6.13	59.21 ± 15.98	56.64 ± 16.17 *	56.10 ± 17.10
α-tocopherol (μM/l)	8.6 ± 3.5	5.8 ± 2.9 *	3.8 ± 1.8 *	4.4 ± 1.7 *
MDA no (μM/l)	0.24 ± 0.06	0.49 ± 0.27	0.80 ± 0.38	0.65 ± 0.21
MDA ox (μM/l)	13.40 ± 11.4	19.65 ± 8.55	21.94 ± 8.13	21.80 ± 7.43

* $P < 0.05$ diabetics versus controls; no, native LDL (μM/l); ox, oxidized LDL (μM/l).

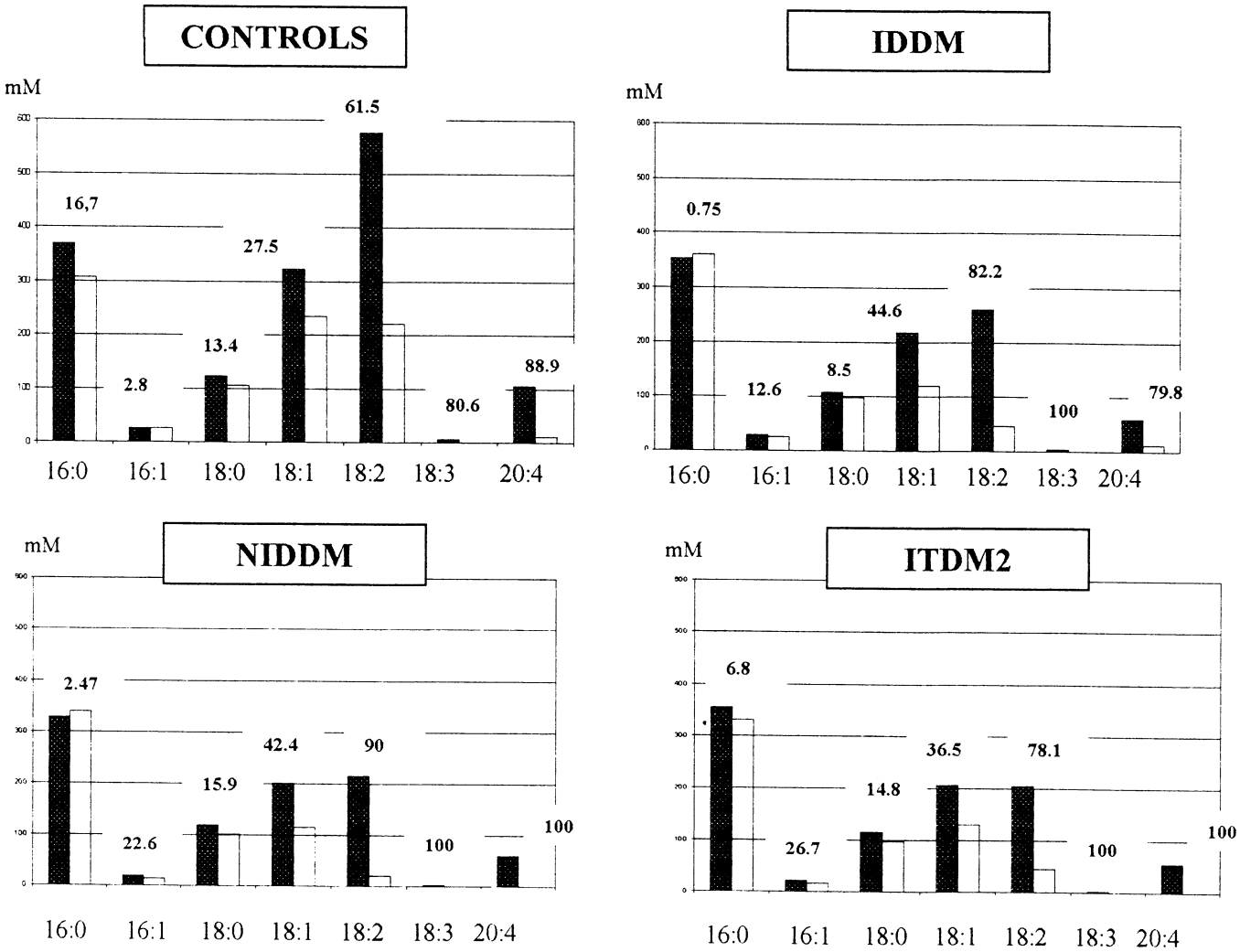

Fig. 2. Fatty acid quantification and percentage of decomposition in native and oxidized LDL in diabetics and controls. Fatty acids — 16:0 palmitic acid; 16:1 palmitoleic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 18:3 linolenic acid; 18:4 linolenic acid; 20:4 arachidonic acid. (■) Native LDL. (□) Oxidized LDL.

2.7. Thiobarbituric acid reactants (TBARS) (μM)

TBARS were determined in plasma with a kit (Sobioda Labs) as described by Richard et al. [6]. Briefly, the plasma sample was boiled under acidic conditions in the presence of thiobarbituric acid (TBA). The released MDA was condensed with TBA and formed TBARS. The concentration of TBARS was determined by measuring the absorbance at 535 nm and calculated using an MDA standard curve.

2.8. α-Tocopherol (μM/l)

α-Tocopherol was measured by HPLC [7]. The chromatographic conditions are as follows: column 3.9 mm × 150 mm symmetry™ C18 (Waters, Milford, MA, USA), injection volume 20 μl, mobile phase methanol 100%, flow rate 0.9 ml/min.

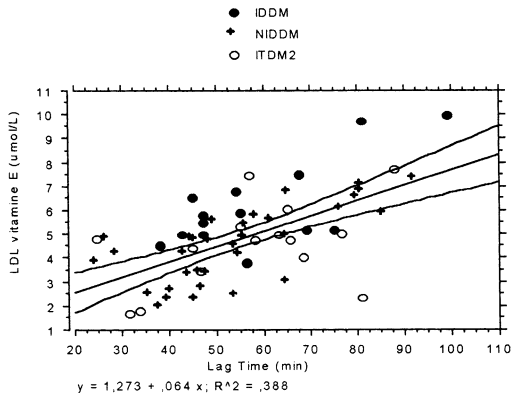

Fig. 3. Correlation between lag time and LDL α-tocopherol in diabetics.

2.9. Statistical analysis

For statistical analysis, the Statview program was used (Abacus concept, Inc., Berkeley, CA, USA). All parameters were given as mean ± SD. Student's *t*-test was used to estimate differences between the groups. The criterion for significance was $P < 0.05$.

3. Results

The absorbance kinetic revealed three phases in healthy and diabetic subjects (Fig. 1):

– *Lag phase:* the absorbance does not increase, indicating that endogenous antioxidants protect LDL from copper oxidation.

– *Propagation phase:* the 234 nm absorption rapidly increases; we have an autocatalytic propagation of the lipid peroxidation process.

– *Decomposition phase:* after reaching the maximum value, the conjugated dienes slowly decrease.

The absorbance curve allows us to calculate the lag time (Fig. 1). Diabetics show a significantly shorter lag time (Table 2), with significantly lower values of LDL content of vitamin E. Polyunsaturated fatty acid decomposition is increased (Fig. 2) in diabetics with elevated MDA content of oxidized LDL (Table 2).

4. Discussion

Studies on LDL oxidizability in diabetes have been conducted mostly on NIDDM and in small cohorts. In this work, we have studied the susceptibility of LDL to in vitro oxidation in three types of diabetes: insulin-dependent diabetes mellitus (IDDM), non-insulin-dependent diabetes mellitus (NIDDM) and insulin-treated diabetes mellitus type 2 (ITDM2).

The LDLs of diabetics are more prone to copper oxidation than controls as lag time is shorter in diabetics. This could be explained by differences in LDL composition; indeed, α-tocopherol is decreased significantly in diabetics; furthermore, we have a negative correlation between α-tocopherol concentration and the lag time in diabetic patients (Fig. 3). Deficiency of the major lipophilic antioxidant in diabetics may result from an enhanced oxidative stress that consumes α-tocopherol.

With regard to MDA, the increased levels observed in these patients in oxidized LDL is in agreement with polyunsaturated fatty acid decomposition after in vitro oxidation; they undergo free radical attack leading to conjugated diene formation and finally to MDA.

For all parameters, ITDM2s have intermediate values between NIDDM and IDDM; insulin medication seems to improve glycemic control and antioxidant defense. The improvement in glycemic control leads to less free radical production; hence oxidative stress is diminished.

Further investigation must be conducted on insulin and LDL oxidizability, because the propensity of LDL to oxidation may contribute to the acceleration of macrovascular disease in diabetes.

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