⟨ Original Article ⟩

Plasma concentrations of oxidized low-density lipoprotein are related to blood glucose concentration but not to total peroxyl radical-trapping potential

Hiroshi Ihara[1], Yuji Oikawa[1], Takashi Kakinoki[1], Yoshikazu Morita[1],
Nobuaki Matsumoto[1], Yoshio Shino[1], Kiyoshi Takamiya[1], Makoto Suzuki[1],
Shin-ichi Katsumata[2], Mariko Uehara[2] and Naotaka Hashizume[3]

Summary   As an index of oxidation of low-density lipoprotein (LDL) by free radicals, plasma concentrations of oxidized LDL (oxLDL) were determined in relation to glycemic markers, serum lipids and lipoproteins, and oxidative markers.   In 89 subjects (41 diabetic patients and 48 healthy subjects), plasma concentrations of oxLDL were positively correlated with blood levels of fasting blood glucose (FBG), hemoglobin A1c (HbA1c), triglycerides (TG), and age, negatively correlated with total bilirubin concentrations, and slightly correlated with ascorbic acid concentrations.   In hyperglycemic subjects (FBG≥126 mg/dL), the blood levels of HbA1c, TG and oxLDL were significantly higher than in normoglycemic subjects (FBG<126 mg/dL).   Total bilirubin and ascorbic acid concentrations decreased in hyperglycemic subjects as compared with normoglycemic subjects.   No significant difference was observed in total peroxyl radical-trapping potential (TRAP) between hyperglycemic subjects and normoglycemic subjects.   In conclusion, plasma concentrations of oxLDL were positively correlated with glycemic levels, but independent of TRAP.

1. Introduction

Oxidation of low-density lipoprotein (LDL) occurs primarily in the intima, in microdomains sequestered from the many plasma antioxidants. LDL enters arterial walls, where it is oxidized by free radicals released by a variety of cells. Oxidized LDL (oxLDL) binds to scavenger receptors on macrophages. The oxLDL is then internalized by the macrophages. Internalized LDL molecules release their cholesterol into macrophages, converting them into foam cells, which are major components of atherosclerotic

---

[1]Department of Laboratory Medicine, Toho University Ohashi Medical Center,
2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
[2]Department of Nutritional Science, Faculty of Applied Bio-Science, Tokyo University of Agriculture,
1-1-1 Sakuragaoka, Setagaya, Tokyo 156-802, Japan

[3]Department of Health and Nutrition, Wayo Women's University,
2-3-1 Konodai, Ichikawa, Chiba 272-8533, Japan

lesions[1]. Oxidation of LDL is reported to be increased in diabetes, in part because of generation of oxygen free radicals during protein glycation (i.e., glycated albumin and glycated hemoglobin) and glucose auto-oxidation[2]. Therefore, it is conceivable that hyper-glycemia of diabetes might result in LDL oxidation. Previously, we have believed that significant degrees of oxidation of LDL do not take place in plasma because of its high antioxidant contents. However, recent studies showed that even very slight degrees of oxLDL can be detectable in the circulating plasma of healthy subjects despite the presence of antioxidants[3]. Therefore, the present study was conducted to determine the relationship between the susceptibility of LDL (i.e., oxLDL concentration in plasma) and antioxidant defenses (i.e., total peroxyl radical-trapping potential: TRAP) in diabetic and healthy subjects in relation to glycemic levels.

2. Subjects and methods

1. Subjects

Fasting venous blood was collected from 48 self-reported healthy subjects (30 males ages 28 to 59 y and 18 females ages 28 to 63 y) and 41 diabetic patients (25 males ages 34 to 84 y and 16 females ages 44 to 84 y). Informed consent was obtained from all subjects, and our study was in compliance with the rules for human experimentation at our institution.

2. Methods

Concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipopro-tein cholesterol (LDL-C), triglycerides (TG), uric acid and total bilirubin in serum were measured by means of enzymatic method on a Hitachi 7600-020S automated analyzer (Hitachi, Tokyo, Japan). Fasting blood glucose (FBG) and hemoglobin A1c (HbA1c) levels were measured by automatic glucose analyzer (Adams Glucose GA-1160, Arkley Co., Kyoto, Japan) and automatic glycohemoglobin analyzer (Adams A1c HA-8160, Arkley Co.), respectively. Concentrations of retinol, α-tocopherol and β-carotene were measured by our developed HPLC methods[4, 5]. Ascorbic acid concentrations in serum

were measured by our automated method[6]. Total sulfhydryl (T-SH) concentrations in serum were measured by the thiocholine method[7, 8, 9]. Total peroxyl radical-trapping potential (TRAP) was calculated stoichimetrically using the experimentally determined efficiency (n value) of each mol of antioxidant to trap a peroxyl radical, e.g. $n = 2.0$ for α-tocopherol[10], $n = 1.7$ for ascorbic acid[11], $n = 1.3$ for uric acid[12], $n = 0.2$ for T-SH[13], $n = 0.21$ for retinol[14], $n = 2.0$ for total bilirubin[15], and $n = 2.0$ for β-carotene[16]. Thus, from the measured concentration of the serum antioxidants: TRAP (μmol/L) = 2.0[α-tocopherol] + 1.7[ascorbic acid] + 1.3[uric acid] + 0.2[T-SH] + 0.21[retinol] + 2.0[total bilirubin] + 2.0[β-carotene]. Plasma concen-trations of oxLDL were measured by a sandwich-type enzyme immunoassay using a murine monoclonal antibody specific for oxidized phosphatidylcholine, and a horseradish peroxidase-labeled goat anti-human antilipoprotein-B IgG (Oxidized Low-density Lipoprotein ELISA, Kyowa Medex Co., Ltd., Tokyo, Japan)[17]. We standardized the ELISA method with copper-oxidized human LDL, and expressed the concentration of oxLDL in terms of the equivalent protein concentration in the copper-oxidized human LDL (4.8 ± 0.2 nmol TBARS/mg protein). For example, 1.0 U/mL oxLDL is equivalent to 250 ng/mL protein in copper-oxidized human LDL. The murine monoclonal antibody did not react with native LDL, malondialdehyde-modified LDL, acetylated LDL, and glycated LDL.

3. Statistics

Statistical analysis was carried out using the Mann-Whitney U-test. Statistical significance was defined as $P < 0.05$.

3. Results

1. oxLDL and other markers

In the overall 89 subjects (41 subjects with diabetes and 48 healthy subjects), plasma concentra-tions of oxLDL were positively correlated with FBG concentrations (r= 0.383, $P < 0.001$: Table 1, column 2 and Fig. 1), HbA1c values (r= 0.362, $P < 0.001$) and TG concentrations (r= 0.241, $P < 0.05$), but negatively

correlated with total bilirubin concentrations (r= -0.311, $P < 0.01$) and slightly correlated with HDL-C and ascorbic acid concentrations. Plasma concentrations of oxLDL were independent of the levels of TRAP (Fig. 1: ●, diabetic males; ○, diabetic females; ▲, healthy males; △, healthy females).

Because there were significant positive correlations between age and oxLDL (r= 0.254, $P < 0.05$), FBG (r= 0.432, $P < 0.001$), or HbA1c (r= 0.439, $P < 0.001$), observed concentrations of oxLDL and FBG and the observed values of HbA1c were adjusted for age, and correlation coefficients were recalculated among them. We obtained the same results, i.e.,

plasma concentrations oxLDL were significantly correlated to FBG (partial correlation coefficient = 0.313, $P < 0.001$) and HbA1c (partial correlation coefficient= 0.268, $P < 0.05$).

2. Influence of glycemic levels

We classified the 89 subjects into two groups according to the FBG levels: one with FBG less than 126 mg/dL (7 diabetic males, 30 healthy males, 6 diabetic females and 18 healthy females) and the other with FBG higher than 126 mg/dL (18 diabetic males and 10 diabetic females). Table 1 shows that mean ($\pm$SD) values of HbA1c, TG and oxLDL were

Table 1   Correlation coefficients between oxLDL and glycemic markers, lipids and lipoproteins, and oxidative markers.

	Correlation coefficient of oxLDL with other markers	FBG levels	
		<126 mg/dL	≥126 mg/dL
N of subjects (M/F)	*89 (55/34)*	*61 (37/24)*	*28 (18/10)*
Age (y)	0.254*	49±13 (28-84)	66±11 (40-83)***
Glycemic markers			
FBG (mg/dL)	0.383***	90±13 (50-118)	204±74 (132-395)***
HbA1c (%)	0.362***	5.7±0.9 (4.3-8.6)	8.5±1.8 (6.0-12.8)***
Lipids and lipoproteins			
TC (mg/dL)	0.088	197±40 (130-300)	204±32 (137-278)
LDL-C (mg/dL)	0.084	109±33 (56-192)	117±28 (69-195)
HDL-C (mg/dL)	−0.137	66±18 (35-130)	59±18 (35-94)
TG (mg/dL)	0.241*	117±82 (34-512)	173±139 (31-723)**
Oxidative markers			
oxLDL (U/mL)		6.55±3.41 (1.52-17.64)	0.83±12.81 (3.03-62.53)**
Uric acid (mg/dL)	0.048	5.3±1.5 (1.8-8.9)	5.2±1.4 (3.0-7.7)
Total bilirubin (mg/dL)	−0.311**	0.83±0.28 (0.40-1.60)	0.69±0.23 (0.30-1.40)*
α-Tocopherol (mg/dL)	−0.002	0.88±0.28 (0.32-1.60)	0.81±0.21 (0.46-1.30)
α-Tocopherol/TC ratio (mg/g)	−0.071	4.47±1.06 (1.93-7.23)	3.96±0.90 (2.46-6.29)
Retinol (μg/dL)	0.06	44±16 (19-112)	43±9 (27-64)
β-Carotene (μg/dL)	−0.023	1.41±0.37 (0.85-2.35)	1.17±0.18 (0.85-1.75)
Ascorbic acid (mg/dL)	−0.130	1.06±0.35 (0.21-2.02)	0.88±0.25 (0.35-1.57)*
T-SH (μmol/L)	0.049	521±56 (411-696)	561±72 (441-726)
TRAP (μmol/L)	0.002	688±110 (474-1008)	659±102 (491-825)

Vales are mean±SD (range).
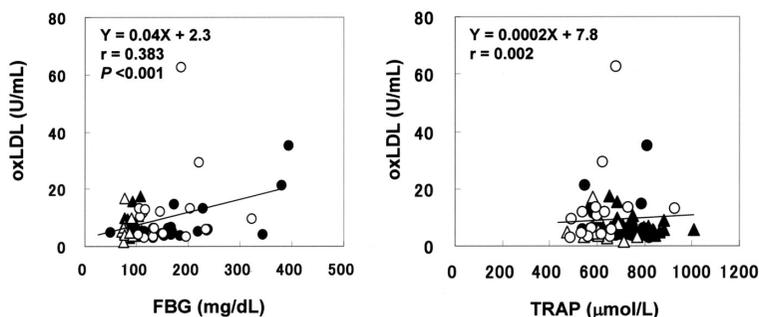*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Fig 1   Correlation of oxLDL with FBG (left) and TRAP (right) in the 89 subjects.
●, diabetic males; ○, diabetic females; ▲, healthy males; △, healthy females.

significantly higher in hyperglycemic subjects (≥126 mg/dL) than those in normoglycemic subjects (<126 mg/dL) (Mann-Whitney U-test: Table 1, columns 3 and 4). However, the mean values of total bilirubin and ascorbic acid concentrations decreased in hyperglycemic subjects. No significant difference was observed in TRAP between hyperglycemic subjects and normoglycemic subjects.

4. Discussion

This study was conducted based on the assumption that plasma levels of oxLDL can be a better index of susceptibility of endothelial cells from free oxygen radicals. In healthy subjects, the antioxidant system defends tissues against free radical attack. Willems et al.[18] showed that autoantibodies to oxLDL present in serum increased with age, but did not correlate with TRAP in well-controlled young type 1 diabetic patients. In accordance with this report we found a significant positive correlation between plasma concentrations of oxLDL and age. However, in contrast to this report that glycemic control and HbA1c levels were not related to autoantibody concentrations and TRAP, respectively, we observed that plasma concentrations of oxLDL were positively correlated with FBG and HbA1c. Genser et al.[19] reported that plasma antioxidant (β-carotene) and TRAP were decreased in patients with Crohn's disease, an inflammatory bowel disease. Even though plasma concentrations of oxLDL were decreased in our subjects (diabetes and healthy subjects), β-carotene, retinol, α-tocopherol, α-tocopherol/TC ratio, uric acid, T-SH, and TRAP remained unchanged. Although antioxidant defenses related to oxidative stress may be the same among diseases, we must take into account the sort of disease in further research.

To our knowledge, this is the first study to describe serum concentrations of total bilirubin and ascorbic acid in hyperglycemic subjects in relation to oxLDL levels. In our study, plasma concentrations of oxLDL were negatively correlated with total bilirubin concentrations and slightly correlated with ascorbic acid concentrations in serum. In these hyperglycemic subjects, blood levels of oxLDL, total bilirubin, and

ascorbic acid decreased as compared with normo-glycemic subjects. Endler et al.[20] reported low serum bilirubin in patients with coronary artery disease (CAD). As suicide antioxidants, bilirubin and ascorbic acid trap newly-formed free radicals to prevent cell damage and further free radical formation. In the above study, Endler et al. hypothesized that bilirubin would scavenge oxidative damage in CAD. Eventually, serum concentrations of bilirubin decreased. In addition, fragmentation of apolipoprotein B-100 in LDL by radical reaction was reported by Hashimoto et al.[21]. They showed that the degree of fragmentation (as a parameter of atherosclerosis) was negatively correlated with serum ascorbic acid concentrations. α-Tocopherol could not protect fragmentation. Contrary to this report, Hoydonck et al.[22] found that supplementation of ascorbic acid to healthy male smokers did not change plasma malondialdehyde and circulating oxLDL concentrations as compared with placebo. No significant differences were observed in the levels of lipids and lipoproteins, oxidative markers, and endothelial markers between smoker and placebo groups. Although ascorbic acid was reported to contribute as a synergist with α-tocopherol, probably by reducing the α-tocopherol radicals to regenerate α-tocopherol at the water-lipid interface of LDL[23], it is difficult to compare with the antioxidant defense of ascorbic acid published in the above studies. However, we can conclude that plasma concentrations of oxLDL are positively correlated with glycemic levels, but independent of TRAP.

References

1) Witztum JL and Steinberg D: Role of oxidized low density lipoprotein in athrogenesis. J Clin Invest, 88: 1785-1792, 1991.

2) Tsai EC, Hirsch IB, Brunzell JD and Chait A: Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes, 43: 1010-1014, 1994.

3) Suzuki T, Kohno H, Hasegawa A, Toshima S, Amaki T, Kurabayashi M and Nagai R: Diagnostic implications of circulating oxidized low density lipoprotein levels as a biochemical risk marker of coronary artery disease. Clin Biochem, 35: 347-353, 2002.

4) Ihara H, Ishigaki H, Shino Y, Hashizume N, Takase M,

Nagao J and Sumiyama Y: Clinical and analytical evaluation of the simultaneous HPLC assay of retinol and alpha-tocopherol. J Nutr Sci Vitaminol, 46: 257-262, 2000.

5) Ihara H, Iahiwata Y and Hashizume N: Metabolism of retinyl oleate in healthy women after vitamin A ingestion. Clin Chem Enzym Comm, 7: 365-372, 1997.

6) Ihara H, Matsumoto N, Shino Y, Aoki Y, Hashizume N, Nanba S and Urayama T: An automated assay for measuring serum ascorbic acid with use of 4-hydroxy-2,2,6,6-tetramethypiperidinyloxy, free radical and o-phenylenediamine. Clin Chim Acta, 301: 193-204, 2000.

7) Koster JF, Biemond P and Swaak AJ: Intracellular and extracellular sulphydryl levels in rheumatoid arthritis. Ann Rheum Dis, 45: 44-46, 1986.

8) Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys, 82: 70-77, 1959.

9) Ishiguro Y, Nakagami Y, Maruta K, Ito Y, Ohashi A, Nagamura Y, Kato M and Sugiyama S: Changes of serum total-SH in hemodialysis patients. J Anal Bio-Sci, 27: 385-390, 2004.

10) Wayner DD, Burton GW, Ingold KU, Barclay LR and Locke SJ: The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochim Biophys Acta, 924: 408-419, 1987.

11) Wayner DD, Burton GW and Ingold KU: The antioxidant efficiency of vitamin C is concentration-dependent. Biochim Biophys Acta, 884: 119-123, 1986.

12) Niki E, Saito M, Yoshikawa Y, Yamamoto Y and Kamiya Y: Oxidation of lipids, XII. Inhibition of oxidation of soybean phosphatidylcholine and methyl linoleate in aqueous dispersions by uric acid. Bull Chem Soc Jpn, 59: 471-477, 1986.

13) Lindeman JH, van Zoeren-Grobben D, Schrijver J, Speek AJ, Poorthuis BJ and Berger HM: The total free radical trapping ability of cord blood plasma in preterm and term babies. Pediatr Res, 26: 20-24,1989.

14) Tesoriere L, Ciaccio M, Bongiorno A, Riccio A, Pintaudi AM and Livrea MA: Antioxidant activity of all-trans-retinol in homogeneous solution and in phosphatidylcholine liposomes. Arch Biochem Biophys, 307: 217-223, 1993.

15) Stocker R, Glazer AN and Ames BN: Antioxidant activity of albumin-bound bilirubin. Proc Natl Acad Sci USA, 84: 5918-5922, 1987.

16) Fujisawa S, Ishihara M and Kadoma Y: Kinetics of the radical scavenging activity of b-carotene-related compounds. SAR QSAR Environ Res, 15: 33-41, 2004.

17) Kohno H, Sueshige N, Oguri K, Izumidate H, Masunari T, Kawamura M, Itabe H, Takano T, Hasegawa A and Nagai R: Simple and practical sandwich-type enzyme immunoassay for human oxidatively modified low density lipoprotein using antioxidized phosphatidyl-choline monoclonal antibody and antihuman apolipopro-tein-B antibody. Clin Biochem, 33: 243-253, 2000.

18) Willems D, Dorchy H and Dufrasne D: Serum antiox-idant status and oxidized LDL in well-controlled young type 1 diabetic patients with and without subclinical complications. Atherosclerosis. 137: Suppl, S61-S64, 1998.

19) Genser D, Kang MH, Vogelsang H and Elmadfa I: Status of lipidsoluble antioxidants and TRAP in patients with Crohn's disease and healthy controls. Eur J Clin Nutr, 53: 675-679, 1999.

20) Endler G, Hamwi A, Sunder-Plassmann R, Exner M, Vukovich T, Mannhalter C, Wojta J, Huber K and Wagner O: Is low serum bilirubin an independent risk factor for coronary artery disease in men but not in women? Clin Chem, 49: 1201-1204, 2003.

21) Hashimoto R, Matsukawa N, Nariyama Y, Ogiri Y, Hamagawa E, Tanaka K, Usui Y, Nakano S, Maruyama T, Kyotani S, Tsushima M and Kojo S: Evaluation of apolipoprotein B-100 fragmentation and cross-linkage in serum as an index of atherosclerosis. Biochim Biophys Acta, 1584:123-128, 2002.

22) Van Hoydonck PG, Schouten EG, Manuel-Y-Keenoy B, van Campenhout A, Hoppenbrouwers KP and Temme EH: Does vitamin C supplementation influence the levels of circulating oxidized LDL, sICAM-1, sVCAM-1 and vWF-antigen in healthy male smokers? Eur J Clin Nutr, 58: 1587-1593, 2004.

23) Sato K, Niki E and Shimasaki H: Free radical-mediated chain oxidation of low density lipoprotein and its syner-gistic inhibition by vitamin E and vitamin C. Arch Biochem Biophys, 279: 402-405, 1990.