Postprandial Low-Density Lipoproteins in Type 2 Diabetes are Oxidized More Extensively Than Fasting Diabetes and Control Samples (44442)

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Abstract. **This study examined the kinetics of low-density lipoprotein (LDL) oxidation in the fasting and postprandial states of diabetic and control subjects to determine if LDL oxidation may contribute to accelerated atherosclerosis in diabetes. We compared *in vitro* oxidation of LDL from 12 control and 13 Type 2 diabetic subjects in the fasting and postprandial states. The extent of oxidation was assessed by length of lag phase, formation of conjugated dienes (CD), lipid peroxides, thiobarbituric acid reactive substances (TBARS), and percentage reduction in free amine groups. Diabetic subjects were significantly older and heavier. Comparisons between control and diabetic subjects in the postprandial state showed that the lag phase was significantly shorter in diabetic subjects than controls ($P = 0.005$), TBARS were significantly higher ($P = 0.006$), and levels of CD were higher at 60, 65, and 70 min ($P < 0.01$). In the fasting state, however, these comparisons were not significant. In diabetic subjects, postprandial samples had a significantly shorter lag phase ($P = 0.003$), higher TBARS ($P = 0.006$), and higher levels of CD at 60, 65 ($P < 0.001$), and 70 min ($P = 0.0013$) compared to fasting samples. Elevated levels of serum triglycerides in diabetic subjects were negatively correlated to lag phase, in fasting ($P = 0.06$) and postprandial states ($P = 0.002$). We conclude that accelerated oxidation of LDL seen in postprandial states in diabetes may be a critical contributor to cardiovascular risks. Elevated levels of serum triglycerides may contribute to the rapid oxidation of LDL seen in diabetic subjects.**
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The association between diabetes and atherosclerosis has long been recognized (1); coronary heart disease is currently the major cause of morbidity and mortality in patients with Type 2 diabetes (2). Aberrations in lipid metabolism are common in diabetes; elevated plasma triglycerides and low high-density lipoprotein (HDL) are the classic dyslipidemia of Type 2 diabetes (3, 4). This combination of lipoprotein abnormalities appears to be highly atherogenic and increases the predisposition of diabetic individuals to atherosclerotic disease (5). Additional evidence suggests that diabetic individuals may have an increased susceptibility to LDL oxidation (6).

The critical role for oxidized LDL in atherosclerosis is reviewed elsewhere (7–10). Formation of fatty streaks within arterial walls is initiated by oxidation of LDL occurring predominantly within the subendothelial space (SES). Lipoprotein lipids and apoproteins are oxidized within the SES as a result of exposure to the free radicals produced by arterial wall cells (11). Oxidized LDL are taken up by macrophages that eventuate as foam cells, precursors of fatty streaks (11, 12).

Twenty years ago Zilversmit (13) suggested that atherosclerosis is a postprandial phenomenon with lipoprotein particles in the postprandial state contributing to atherosclerosis. Because of consumption of regular meals throughout the day, most western people are predominantly in a postprandial state. Most studies of lipoprotein metabolism, however, have focused on the fasting state because it is thought

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to be more reproducible and better-defined baseline for metabolic studies (14). Studies of LDL oxidation have also concentrated on the fasting state although postprandial lipemia may exhibit greater atherogenic potential *via* increases in the postprandial plasma triglyceride levels (15). Levels of triglycerides in the LDL fraction are increased in the postprandial state (14, 16) which, in turn, increases the susceptibility of LDL to oxidative modification (17).

Data on LDL oxidation in the postprandial state are still limited, and kinetics of LDL oxidation in the diabetic condition are not well delineated. The present study was undertaken to determine whether the postprandial LDL from diabetic subjects exhibits alterations that might predispose them to accelerated atherosclerosis as compared to controls. The kinetics of LDL oxidation in fasting and postprandial states of diabetic and control subjects were compared.

These comparisons suggested that oxidation of LDL from the postprandial state of diabetic subjects was accelerated compared to their fasting state. Furthermore, LDL from diabetic subjects in the postprandial state appeared to be oxidized more than postprandial LDL from control subjects. The results suggest that the deviant pattern of LDL oxidation displayed in the postprandial state of diabetic subjects may be a critical contributor to the cardiovascular risks in these individuals.

Materials and Methods

Subjects. Selection criteria for diabetic subjects were as follows: age, 30–70 years; gender, male; BMI, <30 kg/m^2; serum creatinine concentration <177 μM. Inclusion criteria also required serum cholesterol concentration <6.21 m*M* and serum triglyceride concentration <3.4 m*M*. Thirteen poorly controlled type 2 diabetic subjects with glycohemoglobin concentrations >8% and serum glucose concentrations >11.1 m*M* were included in the study. Twelve nondiabetic male subjects served as controls. Control subjects had glycohemoglobin concentrations of <6%, fasting serum glucose concentrations of <6.7 m*M*, serum triglyceride concentrations of <2.26 m*M*, and serum cholesterol concentrations of <6.21 m*M*. Control subjects had no history of diabetes or cardiovascular disease. Subjects for both the groups were not included for these reasons: proteinuria, current cigarette smoking, use of vitamin E supplements, or treatment with glucocorticoids, thyroid hormone, lipid lowering agents, or other drugs known to affect serum lipids. Subjects who met the inclusion criteria at the screening visit were included in the study even though five diabetic subjects subsequently had serum triglyceride values exceeding inclusion criteria.

Test Meal. All subjects were asked to eat a light supper on the day before the study and abstain from alcohol consumption. Following a 12-hr overnight fast, subjects came in as outpatients and had their first phlebotomy of 50 ml of blood. They they were fed a test meal containing 56 g of cornflakes, 250 ml of milk, 250 ml of cream, and 10 g of granulated sugar. Subjects were asked to eat this meal as quickly as possible. The meal provided 960.3 kilocalories and 84.7 g of fat. Six hr after the meal, subjects returned for their second phlebotomy of 50 ml of blood. During the 6 hr after the meal, subjects were instructed to consume only water, coffee, or tea without sugar or cream. The two blood samples were used for the analysis of glucose, glycohemoglobin, and serum lipids, and for lipoprotein isolation. Postprandial studies were scheduled so that one control subject and one or two diabetic subjects had tests on the same day. This ensured that all diabetes samples were processed concurrently with control samples.

Serum Measurements. The Lipid Research Laboratory performed all serum measurements at the VA Medical Center, Lexington, KY. The Abbott Quick-Start glucose kit (Abbott Laboratories, North Chicago, IL) was used to analyze serum glucose concentrations. Glycosylated hemoglobin (HbA_{1c}) concentrations were determined by the Abbott IMx^R glycated hemoglobin test (Abbott Laboratories, normal values, 4.2%–6.4%). The Abbott Spectrum analyzer was used for the analysis of all the serum lipid concentrations (Abbott Laboratories). LDL-cholesterol concentration was determined by the Friedewald formula (18). LDL-cholesterol concentrations were not calculated if serum triglyceride values were >4.5 m*M*.

Isolation of LDL Fraction by Ultracentrifugation. A 50-ml sample of whole blood was collected in tubes containing 0.1% EDTA; plasma was obtained by centrifugation at 2500 rpm for 15 min. Lipoprotein fractions were isolated by a modification of previously described techniques (19). Plasma density was raised to 1.09 g/ml by the addition of KBr. The adjusted plasma was placed in Beckman ultracentrifuge tubes along with saline EDTA (0.1% EDTA) and ultracentrifuged at 50,000 rpm at 4°C for 11.25 hr using a Vti 60 rotor in a Beckman model L8-80 ultracentrifuge. The VLDL-LDL fraction recovered by tube slicing was adjusted to a density of 1.3 g/ml, layered under 27 ml of saline EDTA (0.1% EDTA), and ultracentrifuged at 50,000 rpm for 2.5 hr at 4°C. The LDL fraction was identified visually and obtained by slicing the tubes at the top third portion. Following slicing, each recovered LDL fraction was dialyzed extensively in 4 liters of phosphate buffer saline (PBS) pH 7.4 to remove the EDTA and salts. The dialyzed LDL fractions were stored under N_2 at 4°C until further assays were performed.

Measurement of Oxidation Parameters. Oxidation studies were performed on isolated and dialyzed LDL fractions from all subjects. Methods used for the estimation of the different oxidation parameters are as follows.

In vitro copper oxidation at 37°C. Susceptibility of LDL to *in vitro* oxidation was assessed using copper as a pro-oxidant. Protein content of isolated LDL samples was estimated by the Lowry method (20). Susceptibility of LDL fraction to *in vitro* oxidation was assessed by incubating 50 μg of LDL protein with 5 μM Cu in a final volume of 1 ml of PBS. LDL oxidation was continuously monitored at 37°C for 2 hr, and conjugated dienes (CD), formed as a result of

the oxidation, were measured every 5 min at 234 nm (21) in a temperature-controlled Spectronic Genesys spectrophotometer. After 2 hr, oxidation was stopped by addition of 25 μl of 40 m*M* EDTA and 40 μl of 1 m*M* BHT. The lag phase (minutes) was determined from the oxidation curves obtained, as an intercept of the slope of the oxidation curve and the baseline (21).

Measurement of lipid peroxides and thiobarbituric acid reactive substances formation during LDL oxidation. Formation of lipid peroxides during LDL oxidation was measured spectrophotometrically at 365 nm based on the method described by El-Saadani *et al.* (22). Lipid peroxides formed during LDL oxidation were quantified using the molar absorptivity of iodine measured at 365 nm, which is 2.46×10^4 M^{-1} cm^{-1}. Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Kosugi and Kikugawa (23).

Estimation of the percentage reduction in the free amino groups. The percentage reduction in amino groups as a consequence of derivatization of the lysine residues during oxidation was determined by modification of the method described by Steinbrecher (24). The amount of amino groups remaining following oxidation was estimated based on reactivity of the free amines with trinitrobenzene sulfonic (TNBS) acid. Oxidized LDL, 25 μg was incubated with 1 ml of 4% $NaHCO_3$ and 50 μl of 0.1% TNBS in a shaking water bath at 37°C. After 1 hr, the reaction was stopped with 100 μl of 1 *N* HCl and 100 μl 10% sodium dodecyl sulfate, and absorbance was measured at 340 nm. Native LDL sample, 25 μg, was treated in the same way and from this the percentage reduction in the free amines was determined. Valine was used as a standard.

Electrophoresis. Relative electrophoretic mobility (REM) of all samples was determined by agarose gel electrophoresis as described by Noble (25). A 1% agarose gel was placed in an electrophoresis cell immersed in 0.05 *M* barbital buffer (pH 8.6). Samples were mixed with saturated sucrose and then loaded onto the gel. The gel was run at 90 volts for 45 min, following which it was fixed in 5% acetic acid in 75% ethanol for 30 min. After another 30 min of drying, the gels were stained with Sudan Black until well-stained distinct bands were obtained. Migration of all samples was measured as distance traveled from origin to center of the lipoprotein band. The REM of the oxidized samples was determined by dividing the mobility of modified LDL by mobility of native unoxidized LDL.

A sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to determine purity of isolated apo B-100 LDL fraction. A 4%–20% polyacrylamide gradient gel with 0.1% SDS was cast along with a 3% stacking gel. Samples were boiled with SDS containing β-mercaptoethanol and loaded into wells at a concentration of 5 μg/well. Electrophoresis was carried out at 200 volts (constant voltage) for 45 min. Samples were stained with Coomasie blue stain (0.8%) for 30 min and then destained for 1 hr. Along with LDL fractions, a molecular marker was used as a standard.

Statistical Analysis. All results have been expressed as mean ± SEM. Single factor analysis of variance (ANOVA) test was used to analyze differences statistically between diabetic subjects and controls. The paired *t* test was used for analyses within the group, in the fasting and the postprandial states. Additionally, several parameters were tested for correlations using regression analysis. Significance of comparative tests was determined by *P* values. The *r* values were used to determine correlations between parameters. A two-tailed P value ≤ 0.05 was considered significant. Statistical analyses were performed using Microsoft Excel (Version 5.0, Microsoft, Redmond, WA).

Results

Subject Characteristics. Twelve control subjects and thirteen diabetic subjects were studied. Ten control subjects were Caucasians, one was African-American, and one was Hispanic. Twelve diabetic subjects were Caucasians and one was African-American. Four diabetic subjects had no recognized diabetic complications. Five diabetic subjects had cardiovascular complications, three had hypertension, and five had dyslipidemia. All diabetic subjects were treated by oral hypoglycemic drugs, and none was treated with insulin. At the time of investigation the following medications were being used by diabetic subjects: antianginals (two subjects), nonopoid analgesics (six subjects), digitalis (two subjects), loop diuretics (two subjects), ace inhibitors (two subjects), calcium channel blockers (two subjects), α-blockers (one subject), and anti-inflammatory agents (one subject).

Diabetic subjects were significantly older ($P < 0.001$) than controls and also had a significantly higher BMI ($P = 0.017$). They had very poor glycemic control; serum glucose ($P < 0.001$) and glycohemoglobin concentrations ($P < 0.001$) were significantly higher than values for control subjects (Table I).

Serum Lipids and Lipoprotein Profile. Table I shows serum lipids and lipoprotein concentrations. Although postprandial LDL oxidation measurements were completed in all subjects, we were unable to obtain the postprandial serum lipid and lipoprotein estimations of one control and one diabetic subject. Fasting serum total cholesterol concentrations were 15% higher ($P = 0.022$), and postprandial values were 13% higher ($P = 0.074$) in diabetic subjects than in controls. Fasting LDL-cholesterol concentrations did not differ significantly between control and diabetic subjects. LDL concentrations for two diabetic subjects could not be calculated because serum triglyceride values were >4.5 *M*. Postprandial LDL-cholesterol concentrations were also not calculated because the formula is not applicable to postprandial samples. HDL-cholesterol concentrations were significantly lower in diabetic subjects in both fasting ($P < 0.001$) and fed states ($P = 0.0013$) compared to control subjects. There was no significant change in

Table I. Subject Characteristics

Parameter	Control subjects	Diabetic subjects
Age (years)	44 ± 2.5	61 ± 1.6*
BMI (kg/m^2)	25.3 ± 1.0	28.4 ± 0.8*
Glycohemoglobin (%)	5.3 ± 0.1	9.5 ± 0.4*
Serum glucose (m*M*)	4.95 ± 0.09	11.16 ± 0.83*
Serum cholesterol (m*M*) fasting	4.63 ± 0.18	5.23 ± 0.23*
Serum cholesterol (m*M*) postprandial	4.73 ± 0.26	5.36 ± 0.22
HDL cholesterol (m*M*) fasting	0.99 ± 0.06	0.66 ± 0.05*
HDL cholesterol (m*M*) postprandial	1.01 ± 0.09	0.66 ± 0.05*
Serum triglycerides (m*M*) fasting	1.13 ± 0.14	2.82 ± 0.4*
Serum triglycerides postprandial	1.87 ± 0.21	4.37 ± 0.58*

Values are expressed as Mean ± SEM.
* $P < 0.05$ vs Control

the serum cholesterol and HDL-cholesterol concentrations in the postprandial state of diabetic or control subjects.

The most significant difference between diabetic and control subjects was in the serum triglyceride concentrations. Whereas all diabetic subjects had serum triglyceride concentrations <3.4 m*M* during screening, at the time of the investigation, five subjects had serum triglyceride concentrations >3.4 m*M* (range: 3.5–5.8 m*M*). Because of the limited number of diabetic subjects, LDL oxidation data from the hypertriglyceridemic subjects were included in the analysis. Serum triglyceride concentrations were significantly higher in diabetic than control subjects in both fasting ($P = 0.004$) and postprandial samples ($P < 0.001$). Postprandially, the increments in serum triglyceride concentrations were significant for the control ($P = 0.007$) and diabetic subjects ($P = 0.002$). Postprandial triglyceride concentrations were 65% higher than fasting concentrations in control subjects and 55% higher than in diabetic subjects.

Oxidation Studies. Figure 1 illustrates the LDL oxidation kinetics for the four groups of subjects. Rates of CD formation were similar for fasting and postprandial LDL samples from control subjects although CD values were higher at 120 min for fasting samples. The CD concentration in the fasting samples from diabetic subjects was similar to control fasting samples. There were no differences in the concentration of CD in the postprandial samples from diabetic subjects when compared to postprandial samples from control subjects (Table II). The lag times cannot be estimated from Figure 1 because averaging values changes the slope of the curve; lag times provided in Table II represent the average of individual lag times.

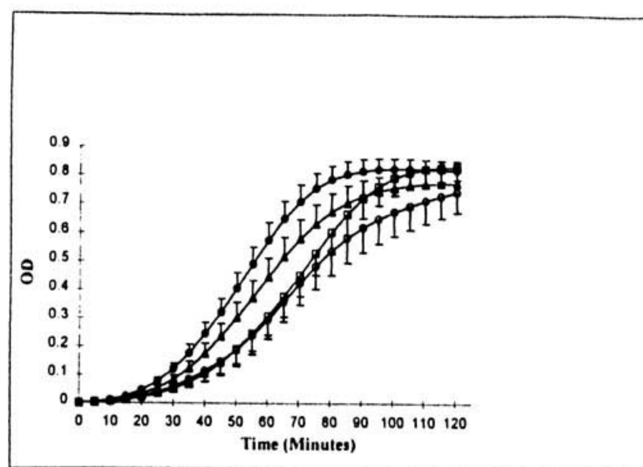

Figure 1. Mean oxidation curves for LDL from control and diabetic subjects. Values represent means, and error bars represent SEM. Symbols are for fasting controls (□), postprandial controls (○), fasting diabetes (▲), and postprandial diabetes (●).

Most CD comparisons in the present study were made at 120 min, at which time LDL oxidation is in the decomposition phase. The rate of CD formation undergoes significant changes in the propagation phase. To account for the differences in the oxidation of LDL from control and diabetic subjects during the dynamic propagation phase, we compared concentrations of CD at 60, 65, and 70 min. In control subjects, these were no significant differences in concentrations of CD between fasting and postprandial samples. In diabetic subjects, postprandial samples had significantly higher values of CD compared to the fasting samples at 60 ($P < 0.001$), 65 ($P < 0.001$), and 70 min ($P = 0.0013$). Comparison between control and diabetic subjects in the fasting state showed that concentrations of CD were similar in fasting samples from diabetic subjects compared to fasting samples from controls at 60, 65, and 70 min. In the postprandial state, however, diabetic subjects had higher levels of CD at 60 min ($P = 0.008$), 65 min ($P = 0.007$), and 70 min ($P = 0.009$) compared to controls (Table II).

In control subjects, there were no significant differences in lag phase between fasting and postprandial samples. In diabetic subjects, the lag phase decreased significantly from 40 min in fasting samples to 33 min in the postprandial samples ($P = 0.003$). Comparison between control and diabetic subjects in the fasting state showed that diabetic subjects had a shorter lag phase compared to controls although this difference was not significant. However, in the postprandial state, lag phase was significantly shorter in diabetic subjects than in controls ($P = 0.005$).

In control subjects, there were no significant differences in lipid peroxide values between fasting and postprandial states. In diabetic subjects, also, there were no significant differences in lipid peroxide values between fasting and postprandial states. Comparison between control and

Table II. Summary of the LDL Oxidation Results

Measurements	CFa*	CPp†	DFa‡	DPp¶
CD (OD)				
60 min	0.31 ± 0.07	0.029 ± 0.08	0.45 ± 0.07	0.57 ± 0.06^{a,c}
65 min	0.38 ± 0.08	0.36 ± 0.08	0.52 ± 0.07	0.65 ± 0.06^{a,c}
70 min	0.45 ± 0.08	0.42 ± 0.09	0.58 ± 0.07	0.71 ± 0.05^{a,d}
120 min	0.83 ± 0.05	0.74 ± 0.07	0.77 ± 0.05	0.82 ± 0.03^{e}
Maximum CD (OD)	0.86 ± 0.05	0.77 ± 0.07	0.82 ± 0.04	0.87 ± 0.03^{e}
Lag phase (minutes)	49.1 ± 4.0	52.9 ± 5.5	40.2 ± 4.1	32.9 ± 3.6^{a,b}
Lipid peroxides (nmoles/mg LDL protein)	675.5 ± 26.4	617.3 ± 66.8	615.1 ± 36.2	642.9 ± 40.1
TBARS (nmoles MDA/mg LDL protein)	89.6 ± 8.2	77.4 ± 7.8	97.1 ± 7.3	107.2 ± 6.2^{a,b}
TNBS (percentage reduction)	33.8 ± 3.5	32.1 ± 4.9	31.1 ± 5.3	32.4 ± 4.6
Relative electrophoretic mobility	1.67 ± 0.06	1.58 ± 0.11	1.80 ± 0.09	1.84 ± 0.08

Values are expressed as Mean ± SEM. $^{a}P < 0.01$ vs CPp; $^{b}P < 0.01$ vs DFa; $^{c}P < 0.001$ vs DFa; $^{d}P = 0.0013$ vs DFa; $^{e}P < 0.05$ vs DFa
* Controls in fasting state; †Controls in postprandial state; ‡Diabetic subjects in fasting state; and ¶Diabetic subjects in postprandial state

diabetic subjects in fasting and postprandial states showed that diabetic subjects had similar levels of lipid peroxides compared to control subjects (Table II).

In control subjects, there were no significant differences in TBARS values between fasting and postprandial states. However, in diabetic subjects, TBARS values were significantly higher in postprandial samples compared to fasting samples ($P = 0.006$). Comparing control and diabetic subjects, TBARS values were slightly but not significantly higher in diabetic subjects than in controls with fasting state ($P = 0.5$) and were significantly higher in the postprandial state ($P = 0.006$). Despite variations in the TBARS observed, the percentage reduction in the free amino groups was very similar in diabetic and control subjects in the fasting and postprandial states.

Isolated LDL fractions were subjected to SDS-PAGE to ascertain the purity of the samples. The unoxidized LDL sample from control and diabetic subjects, fasting and postprandial, represented a single band indicating that the isolated fractions contained only LDL particles (data not shown).

In control subjects, the REM of oxidized fasting samples was similar to the REM of oxidized postprandial samples. In diabetic subjects, as well, the REM of the oxidized fasting and postprandial samples did not differ significantly. Comparison between control and diabetic subjects showed that REM of oxidized samples form diabetic subjects in the fasting and postprandial states were greater than REM of oxidized samples from controls although the differences were not significant (Table II).

Figure 2 illustrates results of regression analysis between serum triglyceride concentrations and lag phase in control and diabetic subjects. In diabetic subjects, serum triglyceride concentrations showed a negative correlation with lag phase in fasting ($r = -0.53$, $P = 0.06$) and postprandial states ($r = -0.80$, $P = 0.002$). In controls, this correlation was absent in both the fasting ($r = 0.01$, $P = 0.97$) and postprandial states ($r = 0.027$, $P = 0.93$). In diabetic subjects, there was a negative correlation between serum cholesterol concentrations and lag phase in both the fasting ($r = -0.41$, $P = 0.16$, not significant) and postprandial states ($r = -0.68$, $P = 0.01$). In control subjects, however, we did not observe a significant correlation between these two parameters in the fasting or postprandial state. We also observed that fasting serum triglyceride concentrations correlated significantly with postprandial values in diabetic subjects ($r = 0.89$, $P < 0.001$). Age was negatively correlated to lag phase in fasting diabetic subjects ($P = 0.034$) but not in postprandial diabetic subjects or control subjects (fasting or postprandial).

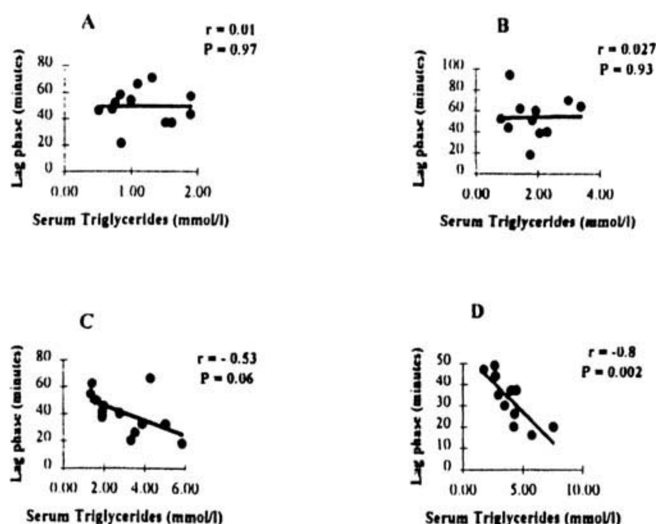

Figure 2. Effects of serum triglyceride levels on lag phase during LDL oxidation. (A) Control fasting state; (B) Control postprandial state; (C) Diabetes fasting state; and (D) Diabetes postprandial state.

Discussion

The kinetic data from our study suggests that oxidation of LDL from diabetic subjects was increased compared to controls and that this difference was of greater significance in the postprandial state. Although prior studies document alterations in composition of LDL from the postprandial state of diabetic subjects, investigations of oxidative susceptibility of LDL are scarce. Our study demonstrated that LDL from diabetic subjects in the postprandial state display increased susceptibility to oxidation. Significant enhance-

ment to oxidation of LDL from diabetic subjects, in the fasting state, has been documented previously by Beaudeux *et al.* (26) and our own laboratory (27). Because we were not able to carefully match control and diabetic subjects for age and BMI, comparisons between these groups have important limitations; the most valid comparisons are the intrasubject comparisons for fasting and postprandial states.

We did not quantitate the lipid and apoprotein concentrations of LDL from these subjects. We hypothesize that higher serum triglyceride levels in diabetic subjects may have contributed to alterations in LDL oxidation. With hypertriglyceridemia, LDL from diabetic individuals is triglyceride-enriched (28) and has profound changes in core and surface composition (29). Such triglyceride-rich LDL particles, in turn, have a proneness to oxidative modification (17). Increased triglyceride content can increase the fluidity of LDL particles (30) and, therefore, accessibility of oxidants to neutral core lipids within the LDL particle. Although we did not assess LDL compositional changes from diabetic or control subjects, we demonstrated a negative correlation between serum triglycerides and lag phase in diabetic subjects in fasting and postprandial states, suggesting that the elevated serum triglyceride levels in diabetic subjects may affect oxidation of LDL. This relationship however was not seen in control subjects.

Our results, consistent with those of Regnstrom *et al.* (17), also revealed a significant inverse correlation between serum cholesterol concentrations and lag phase in the postprandial state of diabetic subjects, suggesting that a rise in the serum cholesterol concentrations may affect the lag phase of LDL oxidation. Oxidation of LDL as related to cholesterol content is indicated to be indirect through a common link to polyunsaturated fatty acids (PUFA) (31). The effect of serum cholesterol levels may be mediated *via* the increased content of PUFA in the cholesterol ester fractions in the LDL cholesterol, which has also been demonstrated to be increased in the LDL fractions of the diabetic subjects (28).

Increased serum triglyceride levels and decreased HDL cholesterol are frequently accompanied by the presence of small dense LDL (32, 33). Serum triglyceride concentrations have, in fact, been reported to be major determinants of the properties of LDL in NIDDM (28, 32, 34). The elevated levels of serum triglycerides in our diabetic subjects could have induced formation of small dense LDL (32, 34, 35). Lewis *et al.* (35) documented that fasting hypertriglyceridemia in NIDDM is an important predictor of postprandial lipids and lipoprotein abnormalities with potential atherogenic effects. We speculate that one such abnormality in the LDL fractions induced by the hypertriglyceridemia in our diabetic subjects may have been a decrease in the particle size, causing acceleration in the oxidative process (36).

Our study provides a basis for further investigations that might furnish important answers about the existence of the phenomenon of accelerated oxidation in diabetic subjects. If these findings are confirmed and extended to other sex and age groups in the diabetic population, LDL oxidation would need to be taken into account as a critical risk factor for coronary heart disease complications in hypertriglyceridemic diabetic subjects.

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