Differences in LDL Oxidizability by Glycemic Status

The Insulin Resistance Atherosclerosis Study

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OBJECTIVE — To investigate differences in LDL oxidizability by glycemic status within the Insulin Resistance Atherosclerosis Study cohort.

RESEARCH DESIGN AND METHODS — LDL oxidizability (lag time and oxidation rate) after exposure to copper was compared among 352 subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), newly diagnosed type 2 diabetes, and known type 2 diabetes.

RESULTS — After adjustment for age, clinic, ethnicity, sex, and smoking status, LDL oxidation rates differed by glycemic status ($P = 0.001$), with a strong trend ($P = 0.0001$) for reduced LDL oxidation rate with increasing extent and duration of glucose intolerance (2,378 ± 54, 2,208 ± 65, 2,145 ± 71, and 2,115 ± 48 arbitrary units [mean ± SE] for NGT, IGT, newly diagnosed type 2 diabetes, and known type 2 diabetes, respectively). Differences in LDL oxidation rate among groups were relatively unaltered by adjustment for lipids and lipoproteins, hypertension, BMI, and waist-to-hip ratio (WHR) and remained significant even after further adjustment for dietary antioxidants and fatty acids, as well as medications. LDL lag times differed marginally by glycemic status ($P = 0.058$), with similar values for NGT, IGT, and newly diagnosed type 2 diabetes (57–60 min) but higher values for known type 2 diabetes (65 ± 2). These differences were eliminated by further adjustment for lipids and lipoproteins, hypertension, BMI, and WHR.

CONCLUSIONS — We found that glycemic status influenced LDL oxidizability, with a paradoxical reduction in LDL oxidizability, as indicated by a lower LDL oxidation rate with increased extent and duration of glucose intolerance. This difference was only slightly attenuated by adjustment for relevant demographic, metabolic, dietary, and pharmacological factors that potentially influence LDL oxidation.

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Cardiovascular diseases are the single most important cause of mortality in Americans (1). Intra-arterial oxidation of LDLs may contribute to atherogenesis (2) and clinical sequelae of atherosclerosis, including coronary heart disease, stroke, and peripheral vascular disease. Individuals with type 2 diabetes experience increased risk of atherosclerosis and clinical cardiovascular disease (3). Previous work suggests that LDL oxidizability may be increased in people with type 2 diabetes (4) and that lipid peroxidation in such individuals is particularly high when glycemic control is poor (5). However, other studies found LDL oxidizability in type 2 diabetic subjects to be either similar (6) or even reduced (7) compared with individuals without diabetes. Data comparing plasma oxidizability among persons with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes are limited (8). Furthermore, it is not known whether duration of diabetes influences LDL oxidizability.

Many of the previous studies of oxidative stress in type 2 diabetes were conducted in relatively small numbers of subjects who were often recruited from medical center clinics. The purpose of this study was to investigate, in a population-based multiethnic cohort including both men and women, whether glycemic status influenced in vitro LDL oxidizability, a measure that may reflect potential for in vivo oxidation. Accordingly, this cohort was selected to include not only subjects with type 2 diabetes and NGT, but also those with IGT and newly diagnosed diabetes. In this cohort, we considered whether LDL oxidizability differs among subjects stratified by glycemic status.

RESEARCH DESIGN AND METHODS

— This report describes an ancillary study conducted in a subsample of 352 male and postmenopausal female participants of the Insulin Resistance Atherosclerosis Study (IRAS) cohort (9). In the original IRAS, individuals with IGT and diabetes were oversampled from ethnically diverse populations (9,10). Exclusions included conditions that would interfere with measurement or interpretation of data for insulin sensitivity or conditions that would limit participation in the study (9). Two of the four IRAS centers participated in this ancillary study. African-American and non-Hispanic

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Abbreviations: IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

white subjects were recruited from the Oakland, CA, site of Kaiser Permanente, a nonprofit health maintenance organization, and Hispanic and non-Hispanic white individuals were recruited from an ongoing population-based study in San Luis Valley, CO (the San Luis Valley Diabetes Study). Data were collected at the 1998–1999 IRAS follow-up visit.

Study variables

A consensus on the best in vivo measure of LDL oxidation is lacking. We used a common measure that is thought to reflect potential for LDL oxidation in vivo: the in vitro susceptibility of LDL to copper and AAPH [azobis(2-amidinopropane)] as oxidizing stressors (11,12). This assessment provides several indices of LDL oxidizability, including lag time (11,12) and propagation rate (11,12), which we used as dependent variables. Lag time denotes the time interval after addition of oxidizing stressor, during which LDL resists oxidation and may reflect endogenous antioxidants in LDL (12). Propagation rate indicates the maximal rate of LDL oxidation, as determined by conjugated diene formation, reflecting intrinsic oxidizability of LDL lipids, once endogenous antioxidants have been depleted (12). Thus, shorter lag times and higher oxidation rates may suggest increased potential for in vivo oxidation. While much attention is given to lag times, the prolonged retention of LDL within arteries of animals (13) (and potentially also human individuals), particularly when atherosclerosis is present (14), suggests that propagation rates may be more worthy of attention.

To prevent oxidation and limit destruction of light-sensitive antioxidants, blood was collected into vacutainers containing EDTA and plasma was transferred to amber vials, overlayered with argon, and stored at 4°C until isolation of LDL. LDL was isolated by ultracentrifugation in the Centers for Disease Control and Prevention–standardized lipid analytical laboratory at Wake Forest University, and protein was measured (11). Before oxidation, EDTA was removed by dialysis for 48 h at 4°C against air-saturated 25 mmol/l phosphate and 0.1 mol/l NaCl, pH 7.2 (PBS), containing 10 μmol/l DTPA (diethylenetriamine-pentaacetic acid) that was continuously sparged with nitrogen gas (15). After equilibration at 37°C, copper sulfate was added to LDL at 10 μg protein/ml in PBS. Copper ion concentrations were adjusted to compensate for the small amount of Cu^{+2} chelated by DTPA (15). The free Cu^{+2} concentration was 3.6 μmol/l. Some assays also included 2 mmol/l $CaCl_2$. Conjugated diene formation was calculated from the increase in absorbance at 236 nm over time (11,12). Because LDL oxidizability determined with 667 μmol/l AAPH provided qualitatively similar results, while variability for propagation rates determined from this assay was greater than for copper-mediated oxidation, such results are not shown.

The primary independent variable of interest was glycemic status (NGT, IGT, newly diagnosed diabetes, and known diabetes). These categories of glycemic status were defined according to World Health Organization criteria using the standard 2-h glucose tolerance test (9). Individuals with diabetes first diagnosed by this glucose tolerance test were considered newly diagnosed diabetic subjects.

Ethnicity was determined by self-report at the 1992–1994 baseline visit; all other measures were determined at the 1998–1999 follow-up visit (9). Postmenopausal status of women was determined by medical history. Smoking status was collected by questionnaire. Blood pressure was measured following a standard protocol, and hypertension status (yes/no) was defined by standard criteria (9).

Plasma triglyceride and plasma total, LDL, and HDL cholesterol were determined in the IRAS central clinical laboratory (16). Dietary intake was assessed by a food frequency questionnaire (17). Nutrient intake was determined with reference to a nutrient database (9). Current medication use was assessed by self-report (9), and individual medications were divided into classes. Separate variables (use/no use) were used to indicate use of each of the following drug classifications: lipid-lowering agents, calcium channel antagonists, β-blockers, ACE inhibitors, aspirin and related compounds, oral hypoglycemic agents, insulin, and female hormones.

Data analysis

ANCOVA, achieved by using PROC GLM from SAS, was used to evaluate the effect of glycemic status on in vitro LDL oxidizability while controlling for both categorical and continuous independent variables in a series of models. When it was necessary to stabilize variance, variables were transformed by taking logarithms or square roots, as appropriate. While transformation of dependent variables enhanced normality and stabilized variance, use of such transformations did not alter overall conclusions. Thus, for simplicity, results for models fitted to untransformed dependent variables are shown.

For models including additional variables, sample sizes differed depending on the number of participants lacking information for these additional variables. Glycemic status, the primary factor of interest, and the other categorical variables (sex, ethnicity, smoking status, and clinic) were considered fixed effects. All potential two-way interactions between fixed effects were considered, and none were found significant. Thus, results are presented for models without such interaction terms.

The first model included glycemic status, with adjustment for age, sex, clinic, ethnicity, and smoking status as potential confounders. Next, we added LDL, HDL, plasma triglyceride, hypertension status, BMI, and waist-to-hip ratio (WHR). WHR was chosen as the measure of central obesity because it was relatively uncorrelated with BMI ($r = 0.09$), while waist circumference and BMI were highly correlated ($r = 0.82$). Subsequently, we added the dietary factors as a group: vitamin E (α-tocopherol equivalents) and vitamin C, β-carotene, and lycopene (in milligrams). Dietary intake of individual nutrients was adjusted for caloric intake using the multivariate nutrient density model (18), which is standard practice (19). Thus, micronutrients were expressed per 1,000 kcal and polyunsaturated fat and oleic acid, the primary sources of dietary monounsaturated fat, as percentages of total calories. To determine the overall relationship between individual nutrients and LDL oxidizability, we summed nutrient intake from foods and food supplements, as suggested (20). Lycopene was transformed by the square root, whereas vitamins E and C and β-carotene were logarithmically transformed. The final model included all of the foregoing and pharmacological variables. Analyses of all subjects, as well as those restricted to the group of 343 participants remaining after excluding those reporting extreme values for dietary intake (consumption of <600 or >5,000

Table 1—*Population demographics for the IRAS LDL oxidation substudy*

Parameter	Overall substudy	San Luis Valley clinic	Oakland clinic
Age (years)	62.3 ± 7.9	62.5 ± 8.2	62.0 ± 7.7
Ethnicity	352	180 (51.1)	172 (48.8)
African American	95 (27.0)	—	95 (55.2)
Hispanics	119 (33.8)	119 (66.1)	—
non-Hispanic white	138 (39.2)	61 (33.9)	77 (44.8)
Sex/hormone status			
Male	174 (49.4)	81 (45.0)	93 (54.1)
Postmenopausal women	178 (50.6)	99 (55.0)	79 (45.9)
Glycemic status			
Normal	105 (29.8)	49 (27.2)	56 (32.6)
IGT	67 (19.0)	35 (19.4)	32 (18.6)
Newly diagnosed diabetes	53 (15.1)	30 (16.7)	23 (13.4)
Known diabetes	127 (36.1)	66 (36.7)	61 (35.5)
Duration since diabetes diagnosis (years)	8.9 ± 7.6	10.1 ± 5.8	7.6 ± 9.0
Total diabetes	180 (51.1)	96 (53.3)	84 (48.8)

Data are mean ± SD or *n* (%).

kcal/day), consistent with the common practice (21), were similar. Thus, we present results for all.

RESULTS — The study population was 27% African American, 34% Hispanic, and 39% non-Hispanic white (Table 1). Approximately half of the participants were men. NGT individuals and those with known diabetes each accounted for about one-third of each clinic population, with the remaining one-third approximately equally split between individuals with IGT and newly diagnosed diabetes. The duration since diagnosis of diabetes averaged 9 years for known diabetic subjects.

Table 2 presents metabolic, behavioral, estimated dietary, and pharmacological variables by glycemic status. Significant linear trends were evident for decreasing plasma total, LDL, and HDL cholesterol and increasing body weight, BMI, WHR, waist circumference, and plasma triglyceride with increasing extent and duration of glucose intolerance ($P < 0.04$ to $P < 0.0001$). Also, total and LDL cholesterol were lower, while BMI, WHR, and waist circumference were higher for people with known diabetes compared with all others ($P < 0.04$ to $P < 0.002$). Nutrient intake did not differ significantly by glycemic status. Prevalence of hypertension and use of ACE inhibitors both generally increased with increasing extent and duration of glucose intolerance (both $P < 0.0001$). A weak trend was evident for progestin use in women. Smoking status and use of lipid-lowering agents, β-blockers, aspirin, and estrogens (among women) did not differ by glycemic status. Among known diabetic subjects, 69% used oral hypoglycemic agents, whereas 16% used insulin. Interestingly, prevalence of aspirin use was very low, and only ~25% as many women used progestins as used estrogens.

We tested the reproducibility of LDL lag times and propagation rates by determining coefficients of variation of blinded repeated measurements for a subset of the participants; these were 10 and 8%, respectively. Table 3 presents lag times and propagation rates for LDL oxidation by glycemic status. After adjustment for clinic, age, sex, ethnicity, and smoking status, lag times differed marginally by glycemic status ($P = 0.058$). Lag time was prolonged in people with known diabetes compared with all others ($P < 0.007$). Further adjustment for LDL and HDL cholesterol, plasma triglyceride, hypertension status, BMI, and WHR (model 2) attenuated differences in LDL lag times among groups such that no significant difference according to glycemic status was apparent. Further adjustment for dietary variables (model 3) and additionally for pharmacological variables (model 4) had little effect on lag times. LDL cholesterol (inversely, $P < 0.0002$, $P < 0.0004$, and $P < 0.0001$ for models 2–4, respectively), BMI (inversely, $P < 0.02$, $P < 0.004$, and $P < 0.0007$ for models 2–4, respectively), dietary vitamin E (directly, $P < 0.0001$, models 3 and 4), and smoking status (inversely, $P < 0.04$, model 4) contributed significantly to variation in lag times.

Table 3 presents propagation rates for LDL oxidation according to glycemic status. After adjusting for age, sex, clinic, ethnicity, and smoking status, propagation rates differed according to glycemic status ($P = 0.0010$), with a trend ($P = 0.0001$) for lower propagation rate for increasing extent and duration of glucose intolerance. Propagation rates were significantly lower for people with known diabetes than for all others ($P \leq 0.05$). Further adjustment for LDL and HDL cholesterol, plasma triglyceride, hypertension status, BMI, and WHR (model 2), as well as dietary variables (model 3), caused only minor numerical changes in propagation rates among groups and attenuated the significance of differences according to glycemic status ($P = 0.038$ and $P = 0.035$ for models 2 and 3, respectively) and the trend ($P = 0.0092$ and $P = 0.0101$ for models 2 and 3, respectively). However, further adjustment for pharmacological variables (model 4) exaggerated differences in propagation rate according to glycemic status ($P = 0.0054$) and the strength of the trend ($P = 0.0012$) for reduced propagation rate with increasing extent and duration of glucose intolerance. Dietary vitamin E was inversely associated with propagation rate ($P < 0.009$ and $P < 0.006$ for models 3 and 4, respectively), independent of all other variables. For model 4, use of oral hypoglycemic agents was directly associated with prop-

Table 2—*Metabolic, behavioral, dietary, and pharmacological variables according to glycemic status for men and postmenopausal women in the IRAS LDL oxidation substudy*

Variable	Normal	IGT	New diabetes	Known diabetes	*P* for trend
n	105	67	53	127	
Metabolic and behavioral variables					
Sex/hormone status					
Male	61 (58.1)	30 (44.8)	20 (37.7)	63 (49.6)	NS
Postmenopausal women	44 (41.9)	37 (55.2)	33 (62.3)	64 (50.4)	
Body weight (kg)	78.6 ± 16.4	81.9 ± 16.4	85.3 ± 21.3	84.1 ± 17.4	0.0099
BMI (kg^2/m)*†	27.4 ± 5.0	29.5 ± 5.7	31.6 ± 6.5	30.8 ± 5.7	0.0001
WHR*†	0.87 ± 0.086	0.87 ± 0.076	0.88 ± 0.078	0.90 ± 0.079	0.0009
Waist circumference (cm)*†	91 ± 13	95 ± 11	99 ± 15	99 ± 12	0.0001
Plasma cholesterol (mg/dl)*	198 ± 36	199 ± 36	196 ± 34	188 ± 36	0.036
LDL cholesterol (mg/dl)*†	127 ± 33	127 ± 31	124 ± 31	115 ± 31	0.004
HDL cholesterol (mg/dl)†	48 ± 17	46 ± 13	42 ± 14	43 ± 14	0.004
Triglyceride (mg/dl)†	116 ± 68	130 ± 64	153 ± 86	151 ± 109	0.0008
Fasting plasma glucose (mg/dl)*†	95 ± 11	103 ± 12	130 ± 39	184 ± 58	0.0001
2-h plasma glucose (mg/dl)*†	108 ± 21	167 ± 17	257 ± 59	349 ± 78	0.0001
HbA_1 (%)*†	5.3 ± 0.3	5.6 ± 0.4	6.3 ± 1.3	8.3 ± 1.9	0.0001
Hypertension	37 (35.2)	34 (50.8)	25 (47.2)	81 (63.8)	0.001
Smoking status					
Never	46 (43.8)	41 (61.2)	29 (54.7)	54 (42.5)	NS
Former	49 (46.7)	22 (32.8)	17 (32.1)	55 (43.3)	
Current	10 (9.5)	4 (6.0)	7 (13.2)	18 (14.2)	
Estimated dietary variables					
Total calories (kcal)	1,959 ± 889	1,825 ± 826	1,913 ± 796	1,808 ± 788	NS
Vitamin E (α-tocopherol equivalents/1,000 kcal)	68 ± 138	61 ± 115	41 ± 93	57 ± 112	NS
Vitamin C (mg/1,000 kcal)	319 ± 627	244 ± 373	215 ± 365	196 ± 245	NS
β-Carotene (mg/1,000 kcal)	2.65 ± 2.74	2.61 ± 2.43	2.38 ± 1.51	2.59 ± 1.96	NS
Lycopene (mg/1,000 kcal)	0.99 ± 0.54	1.00 ± 0.57	1.06 ± 0.58	1.08 ± 0.71	NS
Percent kcal as oleic acid	13.9 ± 4.6	14.2 ± 4.4	14.0 ± 3.3	14.6 ± 4.4	NS
Percent kcal as PUFA	6.9 ± 3.1	6.9 ± 2.4	7.2 ± 2.7	7.4 ± 2.9	NS
Pharmacological variables					
Lipid-lowering drugs	3 (2.9)	7 (10.4)	2 (3.8)	8 (6.3)	NS
β-Blockers	8 (7.6)	9 (13.4)	6 (11.3)	14 (11.0)	NS
Calcium channel blockers	8 (7.6)	5 (7.5)	2 (3.8)	19 (15.0)	0.072
ACE inhibitors	4 (3.8)	7 (10.4)	6 (11.3)	28 (22.0)	0.001
Aspirin use	2 (1.9)	1 (1.5)	1 (1.9)	7 (5.5)	NS
Oral hypoglycemic agents	—	—	0 (0.0)	88 (69.3)	0.001
Insulin	—	—	0 (0.0)	20 (15.8)	0.002
Estrogens (women only)	14 (31.8)	7 (18.9)	13 (39.4)	15 (23.4)	NS
Progestins (women only)	7 (15.9)	2 (5.4)	4 (12.1)	2 (3.1)	0.047

Data are mean ± SD or *n* (%). *$P < 0.05$ for known diabetes vs. all other groups combined; †$P < 0.05$ among groups (ANOVA). PUFA, polyunsaturated fatty acid.

agation rate ($P < 0.03$), and propagation rate was lower for individuals with known diabetes than for all others ($P < 0.03$).

Post hoc comparisons indicated that the relatively shorter lag times and higher propagation rates for newly diagnosed diabetic subjects did not differ significantly from corresponding values for subjects with known diabetes. However, to consider whether this apparently greater LDL oxidizability in newly diagnosed diabetic subjects might be explained by poorer metabolic control in such individuals, we considered fasting and 2-h postprandial plasma glucose concentrations and blood HbA_{1c} (Table 2). However, each of these values was lower for people with newly diagnosed compared with known diabetes.

CONCLUSIONS — Previous data suggest that LDL oxidation contributes to atherosclerosis and cardiovascular disease (2). It is possible that increased LDL oxidation in people with type 2 diabetes, and possibly IGT, contributes to increased risk of cardiovascular disease in these individuals. Arterial cells, including macrophages, can oxidize LDL, and evidence suggests that it is intra-arterial LDL oxidation that is relevant to atherosclerosis (2,12). The joint influences of oxidative capacity of arterial cells, oxidizability of LDL, and LDL concentrations determine

Table 3—*Copper-mediated LDL oxidation according to glycemic status for men and postmenopausal women in the IRAS LDL oxidation substudy*

Adjustment variables	Normal	IGT	New diabetes	Known diabetes	*P* for glycemic status*	*P* for trend†
Estimated lag times (min)						
Model 1: age, EthClin, sex, Smk (0.44)‡	59.7 ± 2.4	58.2 ± 2.9	57.2 ± 3.2	65.4 ± 2.2§	0.058	NS
Model 2: age, EthClin, sex, Smk, *LDL*(−), HDL, Trig, Hyper, *BMI*(−), WHR (0.0037)	59.4 ± 2.5	58.0 ± 2.9	56.7 ± 3.2	63.5 ± 2.2§	NS	NS
Model 3: age, EthClin, sex, Smk, *LDL*(−), *HDL*(−), Trig, Hyper, *BMI*(−), WHR, VitC, *VitE*(+), bCar, Lyc, kcal, Poly, Oleic (0.0001)	59.0 ± 2.4	57.4 ± 2.8	57.3 ± 3.1	63.2 ± 2.2§	NS	NS
Model 4: age, EthClin, sex, *Smk*(−), *LDL*(−), HDL, Trig, Hyper, *BMI*(−), WHR, VitC, *VitE*(+), bCar, Lyc, kcal, Poly, Oleic, ccb, β, ace, lipid, asa, estgn, progst, ohga, insulin (0.0001)	58.6 ± 2.7	56.9 ± 3.1	58.3 ± 3.3	63.3 ± 3.2	NS	NS
Propagation rates (arbitrary units)						
Model 1: age, EthClin, sex, Smk (0.0074)‡	2,378 ± 54	2,208 ± 65	2,145 ± 71	2,115 ± 48§	0.0010	0.0001
Model 2: age, EthClin, sex, Smk, LDL, HDL, *Trig*(−), Hyper, BMI, WHR (0.0091)	2,351 ± 57	2,199 ± 65	2,154 ± 72	2,154 ± 51	0.038	0.0092
Model 3: age, EthClin, sex, Smk, LDL, HDL, Trig, Hyper, BMI, WHR, VitC, *VitE*(−), bCar, Lyc, Poly, Oleic, kcal (0.0127)	2,358 ± 57	2,204 ± 66	2,146 ± 72	2,166 ± 51	0.035	0.0101
Model 4: age, EthClin, sex, Smk, LDL, HDL, Trig, Hyper, BMI, WHR, VitC, *VitE*(−), bCar, Lyc, *Poly*(+), Oleic, kcal, ccb, β, ace, lipid, asa, estgn, progst, *ohga*(+), insulin (0.0178)	2,413 ± 63	2,272 ± 74	2,197 ± 79	2,053 ± 76§	0.0054	0.0012

Data are mean ± SE. **P* for overall effect of glycemic status (ANCOVA); †*P* for linear trend (NGT to IGT to newly diagnosed diabetes to known diabetes or trend in reverse order); ‡*P* for entire model (including all variables listed); §$P < 0.05$ for known diabetes vs. all other groups combined. Italics indicate $P \leq 0.05$ for covariates; (+) and (−) indicate the direction of association. ace, ACE inhibitor use; asa, use of aspirin or related agents; β, β-blocker use; bCar, β-carotene; ccb, calcium channel blocker use; estgn, use of estrogen in any form; EthClin, a combined variable representing clinic and ethnicity; Hyper, hypertension status; insulin, insulin use; lipid, use of lipid-lowering agents; Lyc, lycopene; ohga, use of oral hypoglycemic agents; Oleic, oleic acid; Poly, polyunsaturated fat; progst, use of progestins in any form; Smk, smoking status; Trig, plasma triglyceride; VitC, vitamin C; VitE, vitamin E.

intra-arterial LDL oxidation (2,12,13). Potentially, each of these factors may be altered in type 2 diabetes. It is currently not possible to assess oxidative capacity of arterial cells in living humans. However, one can directly assess in vitro oxidizability of LDL exposed to a standard oxidative stress, as we did in this study. This measure is a commonly used proxy for potential for in vivo oxidation (2,11,12), and LDL lag time has been shown to be increased in individuals with cardiovascular disease (2).

We considered whether glucose intolerance increases in vitro LDL oxidizability. This hypothesis was tested in the IRAS cohort, a population-based study including subjects with NGT as well as those with IGT and both newly diagnosed and known type 2 diabetes. As far as we are aware, this is the first report of the comparison of LDL oxidation among these four categories of glycemic status within a population-based study controlling for the influence of lipids/lipoproteins, dietary antioxidants, dietary fat saturation, and pharmacological agents as well as age,

sex, ethnicity, hypertension, BMI, WHR, and smoking.

The main findings of this study are that in vitro LDL oxidation was not increased in diabetic subjects. In fact, our results indicated lower propagation rates for people with known diabetes compared with all others. While differences in propagation rate were attenuated by adjustment for lipids, lipoproteins, hypertension status, BMI, and WHR, and subsequently for dietary variables, these differences were augmented when pharmacological agents were considered. There was also a significant linear trend for decreasing propagation rate with increasing extent and duration of glucose intolerance.

We found dietary vitamin E to be positively associated with LDL lag time and inversely associated with LDL propagation rate, consistent with previous observations (11,12). Neither β-carotene nor lycopene were associated with LDL lag time or LDL propagation rate, consistent with literature for amounts of these nutrients necessary to influence LDL oxidizability (22,23). We found LDL lag times to be inversely related to plasma LDL cholesterol and smoking, consistent with previous data (2,24).

We observed the expected trends for higher triglyceride and lower HDL cholesterol concentrations with increasing glucose intolerance and of magnitude similar to that for the entire IRAS cohort (25). However, the magnitude of this effect was somewhat less than is often reported for studies of individuals with type 2 diabetes attending hospital-based clinics, which often use subjects recruited from other populations, such as hospital, clinic, or laboratory staff, as comparison groups. In comparison, in our study, all subjects were recruited from the same population, and those with IGT and diabetes were likely to be less ill than those attending hospital clinics, as IRAS excluded individuals requiring insulin at the baseline examination 5 years before this study was conducted.

Our finding of unchanged LDL lag time for diabetic subjects was surprising since alterations in lipids and lipoproteins with increasing glucose intolerance might be expected to increase LDL oxidizability (11,12). Previous studies have reported LDL lag times for type 2 diabetic patients to be reduced (4), similar (6), or even increased (7) compared with individuals without diabetes, while propagation rates were reported to be increased (4) or reduced (6). The reasons for these inconsistencies are not clear. However, all those studies included very small numbers of participants and all compared type 2 diabetic subjects attending hospital clinics with nondiabetic subjects recruited from hospital and clinic staff.

Explanations for reduced propagation rates for people with known diabetes remain to be determined. However, the lower propagation rate for type 2 diabetic subjects is consistent with the reduced LDL size observed for individuals with type 2 diabetes in the IRAS cohort (26), since smaller LDL would provide less oxidizable lipid per unit LDL protein that was included in the assay (11,12). Alternatively, other factors may account for lower LDL propagation rates for people with known diabetes. As adjustment variables, we chose those dietary factors and classes of pharmacological agents that have influenced LDL oxidizability in previous studies. However, we cannot exclude the possibility that other dietary or pharmacological variables that we did not consider may have confounded the relationship between glycemic status and LDL oxidizability.

In this study, LDL oxidizability appeared to be higher in newly diagnosed diabetic subjects than in known diabetic subjects. Poor glycemic control has been associated with increased oxidative stress in individuals with type 2 diabetes (5). In this study, glycemic control, as indicated by each of fasting and 2-h postprandial glucose and HbA_{1c}, was better in individuals with newly diagnosed diabetes, indicating that poorer glucose control could not account for apparently greater LDL oxidizability for these subjects. Future studies will be needed to elucidate the relationship between duration of diabetes and LDL oxidizability.

In summary, we found no evidence for increased in vitro LDL oxidizability for subjects with known diabetes. If one accepts that in vitro LDL oxidizability may reflect potential for in vivo LDL oxidation, one could interpret our results as suggesting that increased LDL oxidation may not be a mechanism by which cardiovascular disease is increased in type 2 diabetes. The surprising observation that in vitro LDL oxidizability was not increased in known type 2 diabetic subjects, and may be even reduced, suggests that one or more correlates of a diagnosis of diabetes, such as treatment of diabetes, metabolic consequences of such treatment, behavioral change, increased duration of diabetes resulting in induction of antioxidant defenses, or other unknown factors, reduce in vitro LDL oxidizability. Finally, while antioxidant treatment reduces early atherosclerosis (2), a review of the cellular effects of antioxidants suggested that once clinically significant atherosclerosis is present, antioxidants may not provide any cardiovascular benefit (2). This notion was recently confirmed by the Medical Research Council/British Heart Foundation Protection Study, which demonstrated that a combined intervention of vitamin E, vitamin C, and β-carotene had no cardiovascular benefit in subjects at high risk of cardiovascular disease, including those with type 2 diabetes (27).

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