Plasma Oxidizability in Mexican-Americans and Non-Hispanic Whites

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Several lines of evidence support an atherogenic role for oxidized low-density lipoprotein (LDL). Previous studies have suggested that although Mexican-Americans have an increased rate of diabetes, obesity, elevated triglyceride levels, and low high-density lipoprotein (HDL) cholesterol levels, their rates of coronary heart disease (CHD) are similar or possibly lower than in non-Hispanic whites. Mexican-Americans have smaller, denser LDL than non-Hispanic whites. On the basis of this latter observation, we postulated that lipid peroxide (LPO) levels would be increased in Mexican-Americans. We examined the oxidizability of plasma in 50 Mexican-Americans and 50 non-Hispanic whites from the San Antonio Heart Study, a population-based study of diabetes and cardiovascular disease, at baseline and after coincubation with a metal-independent system (2′2′-azobis-2-amidinopropane hydrochloride [AAPH]) and a metal-dependent system (Fe^{2+}/H_2O_2) of oxidation. LPO levels were measured by a modified fluorimetric assay. Vitamin E and plasma fatty acid composition were also determined. We found significantly higher LPO levels at baseline and after AAPH coincubation in Mexican-Americans than in non-Hispanic whites (baseline, 2.75 ± .09 *v* 2.07 ± .09 μmol/L, $P < .001$; post-AAPH, 5.49 ± .14 *v* 5.07 ± .04 μmol/L, $P = .037$). However, no significant ethnic differences were seen after coincubation with Fe^{2+}/H_2O_2. Diabetes and cigarette-smoking were also associated with higher LPO levels. Mexican-Americans also had lower levels of vitamin E (the predominant lipid-soluble antioxidant in plasma) than non-Hispanic whites, although these differences only partially explained the differences in susceptibility to oxidation. Plasma fatty acids were similar in Mexican-Americans and non-Hispanic whites, suggesting only small differences in diet composition. We conclude that LPO levels are higher in Mexican-Americans than in non-Hispanic whites, and that these results are only partially related to differences in vitamin E levels.

MEXICAN-AMERICANS differ from non-Hispanic whites in a number of cardiovascular risk factors. Mexican-Americans have a higher incidence of non–insulin-dependent diabetes mellitus (NIDDM),[1] obesity,[2] unfavorable body fat distribution,[3] hyperinsulinemia,[4] and insulin resistance[5] relative to non-Hispanic whites. They also have higher triglyceride and lower high-density lipoprotein (HDL) cholesterol levels than non-Hispanic whites,[6] but a lower prevalence of hypertension.[7] Using logistic regression coefficients from the Framingham Heart Study, Mexican-Americans show a higher predicted risk of coronary heart disease (CHD) than non-Hispanic whites.[8] However, in most studies, Mexican-American men have lower cardiovascular mortality[9] and a lower prevalence of myocardial infarction than non-Hispanic white men.[10,11] In survivors of myocardial infarction, Mexican-Americans may have a higher mortality than non-Hispanic whites.[12] The discrepancy between estimated CHD risk and actual CHD rates suggests that "nontraditional" risk factors for CHD may differ between Mexican-Americans and non-Hispanic whites. We have shown that lipoprotein(a) concentrations are lower in Mexican-Americans than in non-Hispanic whites.[13] In contrast, Mexican-Americans have a preponderance of small, dense low-density lipoprotein (LDL) relative to non-Hispanic whites.[14] However, the increase in small, dense LDL in Mexican-Americans is due to the higher triglyceride levels in this ethnic group.[14] Since smaller, denser LDL is more susceptible to lipid oxidation,[15,16] it is possible that the plasma of Mexican-Americans may be more susceptible to lipid peroxidation.

Recent data have suggested the possibility that oxidized LDL is more atherogenic than ordinary LDL.[17] Oxidized LDL occurs in atherosclerotic lesions in experimental animals,[18] and antibodies to oxidized LDL have been found to correlate with the progression of carotid atherosclerosis.[19] Antioxidant supplementation inhibits the progression of atherosclerosis in experimental animals,[20] and a high intake of vitamin E (an antioxidant) is associated with a lower risk of CHD in both men and women.[21,22]

In this study, we examined plasma lipid peroxide (LPO) concentrations among Mexican-Americans and non-Hispanic whites from the San Antonio Heart Study, a population-based study of diabetes and cardiovascular disease. We also examined the oxidizability of plasma using two model systems (with and without a metal ion). With the first method, plasma was incubated with a free-radical initiator, 2′2′-azobis-2-amidinopropane hydrochloride (AAPH), without a transition metal. In the second system, plasma was incubated with iron (Fe^{2+}) and hydrogen peroxide (H_2O_2) to induce the Fenton reaction.[23] No study has previously examined plasma oxidizability between different ethnic groups. In previous studies, increased plasma oxidizability has been associated with CHD in humans.[24,25] We also examined whether there are ethnic differences in the major lipid-soluble antioxidant in plasma (vitamin E) and plasma polyunsaturated fatty acids ([PUFA] a substrate for lipid oxidation). The rationale for measuring plasma oxidizability is that it offers an evaluation of both prooxidant stress

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and antioxidant defense. This balance could be crucial in determining lipid oxidation.

SUBJECTS AND METHODS

The San Antonio Heart Study is a population-based study of diabetes and cardiovascular disease in Mexican-Americans and non-Hispanic whites that was designed to evaluate the effects of ethnicity and socioeconomic status. From 1979 to 1982 (phase I) and from 1984 to 1988 (phase II), we randomly selected households from several San Antonio, TX, census tracts including low-income (barrio), middle-income (transitional), and high-income (suburban) census tracts.[2,4] All men and nonpregnant women aged 25 to 64 years who resided in the randomly selected households were eligible to participate. Mexican-Americans were defined as individuals whose ancestry and cultural traditions derived from a Mexican national origin.[26] Detailed descriptions of the 1979 to 1982 survey (phase I)[2] and the 1984 to 1988 survey (phase II)[4] have been previously published. This project was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All subjects provided informed consent.

The waist to hip ratio (WHR) was used as a measure of body fat distribution. Body mass index (BMI) was calculated as weight divided by height (in meters) squared. Smoking was assessed by self-report, and subjects were classified as smokers or nonsmokers. CHD was determined by a clinical assessment with an electrocardiogram and by a clinical history of myocardial infarction, cerebrovascular disease, and peripheral vascular disease. None of the subjects were on oral antioxidant supplementation.

At the follow-up examination, blood specimens were obtained after a 12- to 14-hour fast, and a second specimen was obtained 2 hours after administration of a 75-g glucose-equivalent load (Orangedex; Custom Laboratories, Baltimore, MD). Lipid, lipoprotein, and glucose levels were measured using methods previously described.[2,4] These measurements were made in the laboratory of the Division of Clinical Epidemiology, San Antonio, TX. Diabetes mellitus (fasting plasma glucose $\geq$7.8 mmol/L and/or 2-hour post–glucose load plasma glucose $\geq$11.1 mmol/L) was diagnosed according to World Health Organization criteria.[27]

Fasting plasma specimens (collected with EDTA as an anticoagulant) were stored at −70°C for approximately 15 months in San Antonio. They were shipped on dry ice to the laboratory of I. Jialal, MD, in Dallas, TX, for assay of plasma LPO. These samples were not thawed until determinations of plasma LPO were performed. Mexican-Americans (15.0 months) and non-Hispanic whites (14.9 months) were matched for duration of storage of samples. Plasma LPO levels were measured in duplicate in the basal state and after coincubation of plasma in the presence of an aqueous free-radical initiator (50 mmol/L AAPH) that thermally decomposes to produce peroxyl radicals at a constant rate.[23,28] A second model system of coincubation with Fe^{2+} and H_2O_2 was also used to produce LPO.[23] The assay was modified to improve specificity for LPO in the plasma and to prevent interference from bilirubin and sialic acid.[23] The Yagi method has been shown to correlate strongly with high-pressure liquid chromatography methods for determination of LPO levels (r = .85).[29] We have shown previously that the increased plasma oxidation is paralleled by an increase in LDL electrophoretic mobility and a decrease in plasma PUFA, suggesting that lipid peroxidation is being assayed.[23] Furthermore, addition of glucose during the plasma oxidation did not enhance oxidizability. Plasma samples for α-tocopherol were purged with nitrogen and frozen at −80°C until analysis. The measurements were made by reversed-phase high-performance liquid chromatography after extraction with hexane.[30,31] Plasma α-tocopherol was standardized to total plasma lipids.[32] Plasma fatty acids were determined by gas-liquid chromatography after extraction and transmethylation as previously reported.[31,33] An internal standard of C17:0 (NuChek Prep, Elysian, MN) was added to all samples. Total PUFA were calculated as the sum of 18:2, 18:3, and 20.4 fatty acids.

ANCOVA and chi-squared test were performed using SAS statistical software (SAS Institute, Cary, NC). Triglyceride concentrations were log-transformed to improve normality, and were subsequently back-transformed for presentation in tables. Since Mexican-Americans may have increased cholesterol and triglyceride levels relative to non-Hispanic whites and since other studies have indicated that LPO levels are correlated with the total amount of lipid in plasma,[34,35] we computed LPO both per unit volume (micromoles per liter) and also as millimoles per liter of total cholesterol and triglyceride (standardized LPO). Lipid concentrations were not different by ethnicity (Table 1). Since the standardized LPO results were similar to the LPO results per unit volume, we present only the LPO results per unit volume. We present both the unadjusted vitamin E and lipid-standardized vitamin E in this report.

RESULTS

Table 1 shows clinical characteristics of the Mexican-Americans and non-Hispanic whites in this study. Mexican-Americans were significantly more likely to have diabetes than non-Hispanic whites. The percentage of smokers, percentage with CHD, and lipid and lipoprotein levels did

Table 1. Clinical and Metabolic Characteristics (mean ± SE)

Characteristic	Mexican-Americans	Non-Hispanic Whites	P
No. of subjects	50	50	
Men (%)	32	44	.216
Smokers (%)	26	24	.817
Diabetics (%)	40	20	.013
CHD (n)	7	6	.570
Age (yr)	56.8 ± 1.4	52.6 ± 1.8	.068
BMI (kg/m^2)	31.2 ± 1.0	29.7 ± 5.8	.218
WHR	0.932 ± .014	0.932 ± .013	.956
Triglyceride (mmol/L)	2.13 ± 0.09	2.05 ± 1.30	.732
Total cholesterol (mmol/L)	6.04 ± 0.21	5.74 ± 0.18	.290
HDL cholesterol (mmol/L)	1.16 ± 0.02	1.21 ± 0.02	.522
LDL cholesterol (mmol/L)	4.45 ± 0.48	4.11 ± 0.16	.422
Plasma LPO (μmol/L)			
Baseline	2.79 ± 0.08	2.03 ± 0.08	<.001
AAPH	5.51 ± 0.14	5.05 ± 0.14	.019
Fe^{2+}/H_2O_2	7.77 ± 0.31	7.26 ± 0.31	.254
Saturated fatty acids (mmol/L)			
14:0	0.085 ± 0.080	0.760 ± 0.006	.405
16:0	1.826 ± 0.601	1.664 ± 0.457	.142
18:0	0.513 ± 0.019	0.502 ± 0.18	.682
Monounsaturated fatty acids (mmol/L)			
18:4	1.680 ± 0.091	1.510 ± 0.069	.144
PUFA (mmol/L)			
18:2	2.096 ± 0.067	2.018 ± 0.070	.426
18:3	0.461 ± 0.003	0.574 ± 0.003	.012
20:4	0.438 ± 0.018	0.487 ± 0.020	.070
Total (18:2 + 18:3 + 20:4)	2.995 ± 0.077	2.979 ± 0.086	.881
Vitamin E (μmol/L)	21.5 ± 1.0	25.5 ± 1.5	.024
Vitamin E (μmol/mmol/L)*	2.67 ± 0.09	3.39 ± 0.20	.002
PUFA/vitamin E*	0.126 ± 0.004	0.109 ± 0.004	.008

*Lipid-standardized.

not differ between Mexican-Americans and non-Hispanic whites. LPO levels at baseline were significantly higher in Mexican-Americans than in non-Hispanic whites. Whereas lipid peroxidation after AAPH coincubation was significantly higher in the Mexican-American group, after stimulation with Fe^{2+}/H_2O_2, there were not significant differences in Mexican-Americans and non-Hispanic whites. Mexican-Americans had significantly lower vitamin E concentrations and lipid-standardized vitamin E than non-Hispanic whites. There was no significant difference in fatty acids except for lower 18:3 levels in Mexican-Americans. However, the total amount of PUFA (18:2 + 18:3 + 20:4) was not significantly different by ethnic group. The ratio of plasma PUFA to α-tocopherol, an estimate of oxidant stress (substrate) to antioxidant defense, was significantly higher in Mexican-Americans.

Table 2 shows ANOVAs for LPO stratified by ethnicity, gender, smoking status, diabetes, and history of MI.

Table 2. ANCOVA by Ethnicity, Diabetes, Smoking Status, Gender, and Previous Myocardial Infarction (mean ± SE)

	LPO (μmol/L)		
Parameter	Baseline	AAPH	Fe^{2+}/H_2O_2
NIDDM			
MA	2.94 ± 0.12	6.21 ± 0.10	8.21 ± 0.31
NHW	2.16 ± 0.16	5.60 ± 0.12	7.75 ± 0.25
Nondiabetic			
MA	2.64 ± 0.18	5.40 ± 0.12	7.52 ± 0.28
NHW	1.99 ± 0.10	5.00 ± .009	6.81 ± 0.22
P			
NIDDM	.032	.042	.110
Ethnicity	<.001	.032	.242
Smokers			
MA	2.85 ± 0.40	6.01 ± 0.32	8.31 ± 0.31
NHW	2.10 ± 0.32	5.30 ± 0.27	7.19 ± 0.25
Nonsmokers			
MA	1.85 ± 0.20	5.25 ± 0.24	7.20 ± 0.25
NHW	1.20 ± 0.22	4.92 ± 0.19	6.90 ± 0.70
P			
Smoking	.022	.032	.002
Ethnicity	<.001	.042	.240
Men			
MA	2.85 ± 0.14	6.01 ± 0.10	7.71 ± 0.46
NHW	2.35 ± 0.16	5.41 ± 0.12	7.60 ± 0.41
Women			
MA	3.11 ± 0.13	6.11 ± 0.08	8.18 ± 0.37
NHW	2.51 ± 0.16	5.50 ± 0.06	7.50 ± 0.40
P			
Gender	.032	.595	.012
Ethnicity	<.001	.001	.395
Previous MI			
MA	2.93 ± 0.23	5.53 ± 0.15	7.85 ± 0.34
NHW	2.03 ± 0.09	5.22 ± 0.40	7.37 ± 0.90
No MI			
MA	2.77 ± 0.09	5.28 ± 0.37	6.95 ± 0.84
NHW	2.00 ± 0.25	5.03 ± 0.15	7.27 ± 0.34
P			
MI	.681	.872	.509
Ethnicity	<.001	.025	.325

Abbreviations: MA, Mexican-American; NHW, non-Hispanic white; MI, myocardial infarction.

Mexican-Americans continued to have significantly higher LPO levels at baseline and after stimulation by AAPH than non-Hispanic whites even after stratification by NIDDM, gender, smoking status, or previous history of MI. There were no significant ethnic differences in LPO after Fe^{2+}/H_2O_2 stimulation. Subjects with NIDDM had significantly higher LPO levels at baseline and after AAPH stimulation. Women had higher baseline LPO levels than men. Smokers had higher LPO levels than nonsmokers for all three measures of LPO. LPO levels were higher in subjects with a previous myocardial infarction, but these differences were not statistically significant.

Table 3 shows the results of ANCOVA with LPO as the dependent variable. Female gender, Mexican-American ethnicity, cigarette-smoking, and NIDDM significantly predicted higher levels of LPO at baseline. Mexican-American ethnicity, cigarette-smoking, and diabetes were significant predictors of LPO levels after stimulation with AAPH. Cigarette-smoking and female gender were significantly related to higher levels of LPO after stimulation by Fe^{2+}/H_2O_2. We also adjusted further for lipid-standardized vitamin E levels. After these further adjustments, baseline susceptibility to lipid oxidation remained significantly higher in Mexican-Americans than in non-Hispanic whites (2.76 ± 0.9 *v* 2.06 ± 0.9, $P < .001$), but the ethnic difference in lipid susceptibility to oxidation after AAPH stimulation was no longer statistically significant (5.46 ± 0.14 *v* 5.10 ± 0.14, $P = .465$).

DISCUSSION

We have shown in this report that baseline LPO and after stimulation by AAPH were significantly higher in Mexican-Americans than in non-Hispanic whites. These results were unchanged after adjustment for factors such as cigarette-smoking, history of MI, and diabetes. LPO levels after stimulation by Fe_{2+}/H_2O_2 were slightly higher in Mexican-Americans than in non-Hispanic whites, but these differences were not statistically significant. (The variability of LPO levels after Fe_{2+}/H_2O_2 was greater than with the AAPH method [Table 1] and might explain the lack of significance for the ethnic comparison for the former method.) The susceptibility of LDL to oxidizability is governed by three major factors: the amount of oxidizable substrate (principally fatty acids, and especially PUFA), the concentrations of endogenous antioxidants (α-tocopheral, β-carotene, and ascorbate), and the presence of oxidative stress. The PUFA/lipid-standardized α-tocopheral ratio represents the degree of protection afforded by α-tocopheral against oxidative attack. After adjustment for the difference in the antioxidant vitamin E, Mexican-Americans continue to have significantly higher baseline LPO, but the magnitude of the ethnic difference is reduced by approximately 10%. In contrast, the ethnic difference in LPO after AAPH stimulation is no longer statistically significant after adjustment for vitamin E concentrations, suggesting that the ethnic difference in LPO is at least partially due to differences in antioxidant intake. Although we had no dietary questionnaire data, we did measure

Table 3. ANOVA for LPO (mean ± SE)

Parameter	LPO (μmol/L)			Covariates for LPO					
				Baseline		AAPH		Fe^{2+}/H_2O_2	
	Baseline	AAPH	H_2O_2	*P*	Sign	*P*	Sign	*P*	Sign
Mexican-Americans	2.75 ± 0.09	5.49 ± 0.14	7.74 ± .030						
Non-Hispanic whites	2.07 ± 0.09	5.07 ± 0.04	7.29 ± 0.30						
P	<.001	.037	.290						
Gender (F/M)				.035	(+)	.903	(+)	.032	(+)
Age				.605	(+)	.511	(+)	.612	(+)
Smoking status (yes/no)				.017	(+)	.022	(+)	.001	(+)
BMI				.825	(+)	.919	(+)	.240	(+)
WHR				.097	(+)	.566	(+)	.424	(+)
Diabetes (yes/no)				.046	(+)	.021	(+)	.108	(+)
Previous MI (yes/no)				.662	(+)	.873	(+)	.311	(+)

Abbreviations: F, female; M, male.

plasma fatty acid composition, which was similar in both ethnic groups. Increasing vitamin E intake may thus decrease CHD in Mexican-Americans. We have previously reported that Mexican-Americans have a preponderance of small, dense LDL relative to non-Hispanic whites,[14] which is compatible with the present observation, since smaller, denser LDL is more susceptible to oxidation.[15,16]

We also showed increased LPO at baseline and after stimulation with AAPH in diabetic subjects versus nondiabetic subjects. LPO levels after incubation with Fe^{2+}/H_2O_2 were higher in diabetic subjects than in nondiabetic subjects, but the results were not statistically significant. We have previously shown in a separate population that LPO levels after stimulation with AAPH and Fe^{2+}/H_2O_2 were higher in Mexican-American NIDDM subjects than in nondiabetic Mexican-Americans.[36] The difference in the significance of the results after Fe^{2+}/H_2O_2 stimulation between the current study and the previous report[36] may have been due to different populations. However, in each case, LPO levels were higher in diabetic subjects. A number of other studies have shown increases in basal LPO levels in subjects with diabetes.[24,34,35,37-42] Several recent reviews[43,44] have examined possible mechanisms for the increased peroxide levels in diabetic subjects.

We also found significantly higher LPO levels in cigarette smokers than in nonsmokers. LPO levels were higher in subjects with a previous myocardial infarction, as determined by resting electrocardiogram, than in subjects without a myocardial infarction, but these results were not statistically significant. The absence of a relationship to vascular disease as assessed by resting electrocardiogram or clinical history may be due to the low number of subjects and the low sensitivity of this test, leading to low predictive power. We also found higher levels of LPO in women than in men, and also after stimulation with Fe^{2+}/H_2O_2. (No gender difference was observed after AAPH stimulation.) Evans and Orchard[45] had found higher unstimulated LPO levels in women with insulin-dependent diabetes mellitus (IDDM) than in men with IDDM, but found no gender difference in normoglycemic subjects.[45]

One limitation of the present report is that there may be an inconsistency between plasma oxidizability and susceptibility to CHD. Shaish et al[46] showed that ex vivo susceptibility of LDL to AAPH oxidation was not correlated with the extent of lesions in a cholesterol-fed rabbit model of atherosclerosis. In that report, dietary vitamin E supplementation reduced ex vivo LDL oxidizability without a significant effect on the development of atherosclerosis; in contrast, β-carotene did not significantly improve the oxidizability but did reduce atherosclerotic lesions.

In this study, we had hypothesized that Mexican-Americans might have increased susceptibility to oxidation, since they have a larger proportion of small, dense LDL particles[14] and since smaller, denser LDL particles are more susceptible to oxidation.[15,16] However, Bowry et al[47] have shown that HDL is the principal vehicle for circulating plasma lipid hydroperoxides and suggested that HDL lipids may be more rapidly oxidized than those in LDL in vivo. Thus, the higher LPO concentrations reside in the HDL fraction in Mexican-Americans. Although Mexican-Americans have a tendency for lower HDL concentrations than non-Hispanic whites, it is possible that the higher LPO levels in Mexican-Americans are a reflection of a more efficient removal of oxidized lipids by a subclass of HDL rather than an induction of oxidized-particle transport to the arterial wall. If this speculation is correct, then the relative protection from CHD in Mexican-Americans could be explained.

In conclusion, we found significantly higher LPO levels in Mexican-Americans than in non-Hispanic whites. These results are unexplained by differences in cigarette-smoking, MI, diabetes, or diet composition as assessed by free fatty composition. Our results are consistent with previously published data showing increased small, dense LDL in this ethnic group, but do not contribute to explaining the paradoxical lower rate of cardiovascular disease in Mexican-Americans versus non-Hispanic whites. We also observed decreased vitamin E in Mexican-Americans, which is compatible with their susceptibility to oxidation. However, statistical adjustment for vitamin E only partially explained ethnic differences in lipid oxidation. These data suggest that supplementation with vitamin E may reduce the susceptibility to oxidation in this ethnic group.

REFERENCES

1. Haffner SM, Hazuda HP, Mitchell BD, et al: Increased incidence of type II diabetes mellitus in Mexican Americans. Diabetes Care 14:102-108, 1991
2. Stern MP, Rosenthal M, Haffner SM, et al: Sex difference in the effects of sociocultural status on diabetes and cardiovascular risk factors in Mexican Americans: The San Antonio Heart Study. Am J Epidemiol 120:834-851, 1984
3. Haffner SM, Stern MP, Hazuda HP, et al: Upper body adiposity and centralized adiposity in Mexican Americans and non-Hispanic whites: Relationship to body mass index and other behavioral and demographic variables. Int J Obes 10:493-502, 1986
4. Haffner SM, Stern MP, Hazuda HP, et al: Hyperinsulinemia in a population at high risk for non–insulin dependent diabetes mellitus. N Engl J Med 315:220-224, 1986
5. Haffner SM, Stern MP, Dunn JF, et al: Diminished insulin sensitivity and increased insulin response in non-obese, non-diabetic Mexican Americans. Metabolism 39:842-847, 1990
6. Haffner SM, Stern MP, Hazuda HP, et al: The role of behavioral variables, and fat patterning in explaining ethnic differences in serum lipids and lipoproteins. Am J Epidemiol 123:830-839, 1986
7. Haffner SM, Mitchell BD, Stern MP, et al: Decreased prevalence of hypertension in Mexican Americans. Hypertension 16:225-232, 1990
8. Mitchell BD, Stern MP, Haffner SM, et al: Risk factors for cardiovascular mortality in Mexican Americans and non-Hispanic whites: The San Antonio Heart Study. Am J Epidemiol 131:423-433, 1990
9. Stern MP, Bradshaw BS, Eifler CW, et al: Secular decline in death rates due to ischemic heart disease in Mexican Americans and non-Hispanic whites in Texas, 1970-1980. Circulation 76:1245-1250, 1987
10. Mitchell BD, Hazuda HP, Haffner SM, et al: Myocardial infarction in Mexican Americans and non-Hispanic whites: The San Antonio Heart Study. Circulation 83:45-51, 1991
11. Rewers M, Shetterly SM, Baxter J, et al: Prevalence of coronary heart disease in subjects with normal and impaired glucose tolerance and non–insulin dependent diabetes mellitus in a biethnic Colorado population. The San Luis Valley Diabetes Study. Am J Epidemiol 135:1321-1330, 1992
12. Orlander PR, Goff DC, Morrissey M, et al: The relationship of diabetes to the severity of acute myocardial infarction and post-myocardial infarction survival in Mexican Americans and non-Hispanic whites. The Corpus Christi Heart Project. Diabetes 43:897-902, 1994
13. Haffner SM, Gruber KK, Morales PA, et al: Lipoprotein(a) concentrations in Mexican Americans and non-Hispanic whites: The San Antonio Heart Study. Am J Epidemiol 136:1060-1068, 1992
14. Haffner SM, Mykkänen L, Valdez RA, et al: LDL size and subclass pattern in a biethnic population. Arterioscler Thromb 13:1623-1630, 1993
15. de Graaf J, Hak-Lemmers HLM, Hectors MPC, et al: Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 11:298-306, 1991
16. Tribble DL, Holl LG, Wood PD, et al: Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 93:189-199, 1992
17. Witztum JL, Steinberg D: Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785-1792, 1991
18. Ylä-Herttuala S, Palinski W, Rosenfeld ME, et al: Evidence for the presence of oxidatively modified LDL in atherosclerotic lesions of rabbit and man. J Clin Invest 84:1086-1095, 1989
19. Salonen JT, Ylä-Herttuala S, Yamamoto R, et al: Autoantibody against oxidized low density lipoprotein and progression of carotid atherosclerosis. Lancet 339:883-887, 1992
20. Kita T, Nagano Y, Yokode M, et al: Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci USA 84:5928-5931, 1987
21. Stampfer MJ, Hennekens CH, Manson JE, et al: Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328:1444-1449, 1993
22. Rimm EB, Stampfer MJ, Ascherio A, et al: Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 328:1450-1456, 1993
23. Agil A, Fuller CJ, Jialal I: Susceptibility of plasma to ferrous iron/hydrogen peroxide–mediated oxidation: Demonstration of a possible Fenton reaction. Clin Chem 41:220-225, 1995
24. Yagi K: Lipid peroxides and human diseases. Chem Phys Lipids 45:337-351, 1987
25. Stringer MD, Görög PG, Freeman A, et al: Lipid peroxides and atherosclerosis. Br Med J 298:281-284, 1989
26. Hazuda HP, Comeaux PJ, Stern MP, et al: A comparison of three indicators for identifying Mexican Americans in epidemiologic research: Methodological findings from the San Antonio Heart Study. Am J Epidemiol 123:96-112, 1986
27. World Health Organization Expert Committee on Diabetes Mellitus. Second Report. Geneva, Switzerland, World Health Organization, 1982, pp 125-143
28. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377-6381, 1989
29. Therasse J, Lemonnier F: Determination of plasma lipoperoxides by high performance liquid chromatography. J Chromatogr 413:237-241, 1987
30. Arnaud J, Fortis I, Blachier S, et al: Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography. J Chromatogr 572:103-116, 1991
31. Schmuck A, Fuller CJ, Devaraj S, et al: Effect of aging on susceptibility of low-density lipoproteins to oxidation. Clin Chem 41:1628-1632, 1995
32. Thurnham D, Davies J, Crump B, et al: The use of different lipids to express serum tocopherol: Lipid ratios for the measurement of vitamin E status. Ann Clin Biochem 23:514-520, 1986
33. Lepage G, Roy CC: Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114-120, 1986
34. Nishigaki I, Hagihara M, Tsunekawa H, et al: Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. Biochem Med 25:373-378, 1981
35. Mooradian AD: Increased serum conjugated dienes in elderly diabetic patients. J Am Geriatr Soc 39:571-574, 1991
36. Haffner SM, Agil A, Mykkänen L, et al: Plasma oxidizability in subjects with normal glucose tolerance, impaired glucose tolerance and NIDDM. Diabetes Care 18:646-653, 1995
37. Sato Y, Hotta N, Sakamoto N, et al: Lipid peroxide level in plasma of diabetic patients. Biochem Med 21:104-107, 1979
38. Kaji H, Kurasaki M, Ito K, et al: Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non–insulin-dependent) diabetic women. Klin Wochenschr 63:765-768, 1985
39. Jennings PE, Jones AF, Florkowski CM, et al: Increased

diene conjugates in diabetic subjects with microangiopathy. Diabetic Med 4:452-456, 1987

40. MacRury SM, Gordon D, Wilson R, et al: A comparison of different methods of assessing free radical activity in type 2 diabetes and peripheral vascular disease. Diabetic Med 10:331-335, 1993

41. Altamore E, Vendemaille G, Chicco D, et al: Increased lipid peroxide in type 2 poorly controlled diabetic patients. Diabete Metab 18:264-271, 1992

42. Velazquez E, Winocour PH, Kesteven P, et al: Relation of lipid peroxides to macrovascular disease in type 2 diabetes. Diabetic Med 8:752-758, 1991

43. Chisolm GM, Irwin KC, Penn MS: Lipoprotein oxidation and lipoprotein-induced cell injury in diabetes. Diabetes 41:61-66, 1992 (suppl 2)

44. Lyons TJ: Glycation and oxidation: A role in the pathogenesis of atherosclerosis. Am J Cardiol 71:26B-31B, 1993

45. Evans RW, Orchard TJ: Oxidized lipids in insulin-dependent diabetes mellitus: A sex-diabetes interaction? Metabolism 43:1196-1200, 1994

46. Shaish A, Daugherty A, O'Sullivan F, et al: Beta-carotene inhibits atherosclerosis in hypercholesterolemic rabbits. J Clin Invest 96:2075-2082, 1995

47. Bowry VW, Stanley KK, Stocker R: High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. Proc Natl Acad Sci USA 89:10316-10320, 1996