

Apolipoprotein E Polymorphism and Insulin Levels in a Biethnic Population

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OBJECTIVE — To study the association of apolipoprotein E (apoE) polymorphism with important cardiovascular risk factors other than cholesterol levels, such as insulinemia and insulin resistance.

RESEARCH DESIGN AND METHODS — In this report, we study the association of three major apoE phenotypes (apoE 3/2, apoE 3/3, and apoE 4/3) with indicators of insulin resistance such as fasting insulin, glucose, and lipid levels in 320 nondiabetic Mexican-Americans and non-Hispanic whites from San Antonio, TX.

RESULTS — The two ethnic groups differed in the frequencies of the three major apoE phenotypes. However, the associations of these phenotypes with lipid and insulin levels were similar in both ethnic groups and in both sexes. Compared with the other two major apoE phenotypes, the apoE 3/2 phenotype was associated with lower levels of total and low-density lipoprotein cholesterol and lower levels of fasting and 2-h postload insulin.

CONCLUSIONS — In addition to the association with cholesterol levels, the variability at the apoE locus may be associated with a much broader set of metabolic factors that relate to insulin resistance.

Apolipoprotein E (apoE) participates in the receptor-mediated clearance of blood lipids such as cholesterol, which is considered a major risk factor for

coronary artery disease (CAD). It has been estimated that the variability at the apoE gene locus accounts for up to 6% of the variation in risk for CAD in North

America (1). This locus lies on chromosome 19, and its three common alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, code for the isoforms apoE2, apoE3, and apoE4, respectively (2,3). The frequencies of these isoforms vary from population to population, but apoE3 always shows the highest frequency ($\geq 49\%$) and apoE2 the lowest ($\leq 15\%$) (4). Population studies have also shown that compared with the isoform apoE3, the isoform apoE2 is associated with low and the isoform apoE4 with high levels of plasma low-density lipoprotein (LDL) cholesterol (5,6). Similarly, compared with that of diabetic patients carrying the isoform apoE3, the cardiovascular risk profile seems better in diabetic patients carrying the isoform apoE2 and worse in those carrying the isoform apoE4 (7–9).

Although the relationship between the apoE polymorphism and total and LDL cholesterol has been extensively studied, there is a lack of studies analyzing the association between this polymorphism and other important CAD risk factors, such as insulinemia and insulin resistance. A recent study suggests that such associations are possible, since the well-established positive relationship between insulin and triglyceride levels was not found among normoglycemic women carrying the isoform apoE4 (10).

In this report, we describe the association of the apoE polymorphism with metabolic indicators of insulin resistance, namely, fasting insulin, glucose, and lipid levels in nondiabetic individuals from two ethnic groups with contrasting levels of risk for type II diabetes: Mexican-Americans (high risk) and non-Hispanic whites (low risk).

RESEARCH DESIGN AND METHODS

RESEARCH DESIGN AND METHODS — The San Antonio Heart Study is a longitudinal population-based study of diabetes and cardiovascular risk factors in 3,302 Mexican-Americans and 1,877 non-Hispanic whites, 25–64 years of age at entry, who were enrolled in two phases (1979–1982, phase I, and 1984–

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ANCOVA, analysis of covariance; apoE, apolipoprotein E; BMI, body mass index; CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; STR, subscapular-to-triceps skinfold ratio; VLDL, very-low-density lipoprotein; WHR, waist-to-hip circumference ratio.

1988, phase II). Detailed descriptions of these surveys have appeared elsewhere (11,12). Mexican-Americans were defined as individuals whose ancestry and traditions are derived from a Mexican national origin (13).

An 8-year follow-up of the phase I cohort has been completed, with 80.8% ($n = 1,685$) of the surviving subjects being re-examined using procedures identical to those used at the baseline examination. The follow-up to the phase II cohort is currently in progress.

For each individual, blood samples were obtained after a 12-h fast and 2 h after the administration of a 75-g oral glucose equivalent load (Glucola, Ames, Elkhart, IN). Glucose was measured by a glucose oxidase method. Insulin was measured by a solid phase radioimmunoassay (Diagnostic, Los Angeles, CA) (14). Serum cholesterol was determined by the cholesterol oxidase method using an Abbott VP autoanalyzer (15), and triglyceride level was measured after hydrolysis of the glyceride using an enzymatic method for glycerol determination (16). High-, low-, and very-low-density lipoproteins (HDL, LDL, and VLDL) were measured by the procedures used by the Lipid Research Clinics (17). Lipoprotein concentrations are expressed in terms of their cholesterol concentrations. Very-low-density lipoprotein was removed by ultracentrifugation in those specimens having a total serum triglyceride concentration >300 mg/dl. Otherwise, VLDL cholesterol was estimated as total triglyceride divided by five (17). LDL cholesterol was estimated as total cholesterol minus the sum of HDL and VLDL cholesterol. We measured HDL cholesterol after precipitation of β -lipoproteins by the dextran sulfate method (18). Diabetes was diagnosed according to the criteria of the World Health Organization (19). Subjects who were under treatment with either oral antidiabetic agents or insulin were also considered to have diabetes regardless of their plasma glucose levels.

The anthropometric measurements, waist and hip circumferences

Table 1—ApoE phenotype and allele frequencies by ethnicity

	Mexican-Americans		Non-Hispanic whites	
	<i>n</i>	Frequency	<i>n</i>	Frequency
ApoE phenotype				
2/2	1	0.35	0	0.00
4/2	0	0.00	3	2.10
4/4	3	1.06	4	2.80
3/2	13	4.59	21	14.69
4/3	39	13.78	24	16.78
3/3	227	80.21	91	63.64
ApoE allele				
2	—	0.026	—	0.084
3	—	0.894	—	0.794
4	—	0.080	—	0.122

Between the two groups, $P < 0.001$ for the difference in phenotype frequencies and $P < 0.001$ for the difference in allele frequencies.

(phase II only), weight, height, and subscapular and triceps skinfolds, were made using standard procedures (20). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. The ratios of subscapular-to-triceps skinfold (STR) and waist-to-hip circumference (WHR) were used as indexes of central and upper body adiposity, respectively. Skin reflectance was measured at the inner aspect of the upper arm using a portable spectrophotometer. We also considered the effect of alcohol intake and physical activity. The distribution of physical activity and alcohol intake did not differ by apoE isoform, and the addition of these variables as covariates did not change any of the results. Therefore, we did not consider these variables further in this report.

The subjects in this report ($n = 426$) are from the first two census tracts of the phase II follow-up, a middle-income neighborhood (50% Mexican-American and 50% non-Hispanic white), and a low-income neighborhood (100% Mexican-American).

The apoE phenotypes were determined from small amounts (10 μ l) of whole plasma using isoelectric focusing followed by immunoblotting (21). Briefly, polyacrylamide gels were pre-

pared on 200×260 glass plates. The cathode and anode solutions were 1.0 mol/l NaOH and 1.0 mol/l H_3PO_4 , respectively. LKB 2197 power-supply and LKB 2117 Multiphor II electrophoresis units (LKB, Gaithersburg, MD) were used for electrofocusing at 2,000 V. Fifteen-minute prefocusing was carried out before applying the samples. A 4×10 mm piece of Whatman (Clifton, NJ) 3MM chromatography paper (sample wick) was dipped into each sample tube and, after blotting, was placed on the gel 5 mm from the cathode (80 samples/plate). Initial focusing was carried out for 30 min with voltage increasing from 1,000 to 1,600 V. The sample wicks were removed after 30 min, and subsequent focusing was conducted for an additional 1.5 h. After electrophoresis, the protein was transferred by simple diffusion using a 0.45- μ m (pore size) nitrocellulose membrane (BA-S85; Schleicher & Schuell, Keene, NH). The immunoblotting was completed as described by Kamboh et al. (22).

Subjects with diabetes ($n = 88$) and subjects whose diabetic status was unknown ($n = 7$) were excluded. An additional group of subjects ($n = 11$) with apoE phenotypes of low frequency was also excluded. These phenotypes were

Table 2—Characteristics of the study subjects by ethnic group

	Ethnicity		P value
	Mexican-Americans	Non-Hispanic whites	
n	203	117	—
Age (years)	48.6 ± 0.75	52.1 ± 1.23	0.045
Male (%)	36.5	47.9	0.045
ApoE phenotype (%)			
3/2	4.9	17.1	—
3/3	82.8	64.9	—
4/3	12.3	18.0	<0.001
BMI (kg/m ²)	30.5	27.9	<0.001
WHR	0.923 ± 0.006	0.925 ± 0.007	0.853
STR	1.38 ± 0.031	1.21 ± 0.041	0.001
Skinfolds (mm)			
Subscapular	27.0 ± 0.6	25.3 ± 0.8	0.095
Triceps	22.3 ± 0.6	23.0 ± 0.8	0.448
Circumferences (mm)			
Waist	982.6 ± 12.9	980.1 ± 17.2	0.908
Hips	1,062.3 ± 10.6	1,053.6 ± 14.1	0.623
Fasting insulin (μU/ml)	9.8 (8.5–11.8)	5.8 (4.9–7.2)	<0.001
2-h insulin (μU/ml)	63.5 (54.8–73.0)	34.8 (29.3–42.6)	<0.001
Fasting glucose (mg/dl)	91.2 ± 0.70	88.8 ± 0.92	0.045
2-h glucose (mg/dl)	118.1 ± 2.33	112.4 ± 3.06	0.149
Triglycerides (mg/dl)	145.7 (135.7–156.9)	138.4 (127.0–153.7)	0.412
HDL cholesterol (mg/dl)	43.5 ± 0.88	44.2 ± 1.16	0.664
Total cholesterol (mg/dl)	221.9 ± 3.08	221.4 ± 4.04	0.914
LDL cholesterol (mg/dl)	144.9 ± 2.88	145.6 ± 3.75	0.878

Data are n means ± SE or value (95% confidence interval). Fasting insulin, 2-h postload insulin, and triglycerides were back transformed from log transformation. Values are for nondiabetic subjects only and are adjusted for age and sex.

apoE 4/4, apoE 4/2, and apoE 2/2. Their frequencies are shown in Table 1. However, the analyses were also performed after including the 11 subjects with low-frequency phenotypes and grouping all phenotypes as follows: the E2 group, which included the apoE 2/2 and the apoE 3/2 phenotypes; the E3 group, which included the apoE 3/3 phenotypes only; and the E4 group, which included the apoE 3/4 and apoE 4/4 phenotypes. The results were identical to the ones in which the rare phenotypes were excluded. For simplicity, we report here the results without the low-frequency phenotypes. Hence, the analyzable sample size of this study is 320 subjects, 203 Mexican-Americans and 117 non-Hispanic whites. The frequencies of the three major apoE phenotypes did not change signifi-

cantly in either ethnic group after all the exclusions were made (Tables 1 and 2).

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All subjects gave informed consent.

Statistical analysis

Analysis of covariance (ANCOVA) was used to test for differences between mean values across the three major apoE phenotypes. Chi-squared tests were used to compare differences in frequencies. All statistical analyses were performed using software developed by the SAS Institute (23). Triglyceride and fasting and 2-h insulin concentrations were log-transformed for statistical analysis and then back-transformed for presentation in the

tables. Isoform and phenotypic frequencies were determined by counting. Only three subjects had Apo 4/2 and only five had 4/4; thus they were excluded from this report. We performed analysis of variance with interaction terms of apoE × sex and apoE × ethnicity. These interaction terms were not statistically significant ($P > 0.20$); therefore, ethnic groups and sexes were pooled in Table 5.

RESULTS — The total frequencies of the three apoE isoforms were significantly different between Mexican-Americans and non-Hispanic whites (Table 1). There were no significant differences by sex in the frequencies of these major apoE phenotypes (data not shown). Characteristics of the study subjects, by ethnic group, are presented in Table 2. Several of these de-

Table 3—Mean values of metabolic characteristics by ethnic group and apoE phenotype adjusted for age, sex, and BMI

	Mexican-American				Non-Hispanic white			
	ApoE phenotype			P value	ApoE phenotype			P value
	3/2	3/3	4/3		3/2	3/3	4/3	
n	10	168	25	—	20	76	21	—
Fasting insulin (μ U/ml)	4.8	10.1	11.5	0.010	4.3	6.5	5.5	0.423
2-h insulin (μ U/ml)	35.7	61.6	94.2	0.023	21.5	38.2	40.8	0.065
Fasting glucose (mg/dl)	92.5	91.0	89.2	0.598	84.7	89.8	92.5	0.028
2-h glucose (mg/dl)	115.3	116.7	121.7	0.719	102.3	115.4	114.3	0.304
Circumferences (mm)								
Waist	907.5	980.6	1001.5	.125	982.8	987.7	978.8	0.899
Hips	964.5	1067.8	1078.3	.014	1051.2	1052.0	1043.8	0.809
Skinfolds (mm)								
Subscapular	25.7	27.4	27.7	.737	24.7	25.2	22.9	0.269
Triceps	22.9	22.7	23.1	.973	0.928	0.936	0.936	0.188
WHR	0.914	0.918	0.924	0.910	0.928	0.936	0.936	0.892
STR	1.23	1.36	1.38	.661	1.16	1.24	1.29	0.605
Triglycerides (mg/dl)	102.4	144.2	163.4	0.077	141.6	132.8	179.9	0.020
HDL cholesterol (mg/dl)	48.2	43.7	41.7	0.427	45.5	43.8	41.6	0.477
Total cholesterol (mg/dl)	193.7	220.8	231.2	0.073	210.2	226.7	224.5	0.319
LDL cholesterol (mg/dl)	113.6	144.0	153.9	0.037	131.4	153.7	140.1	0.063
Skin reflectance (%)	25.4	28.4	28.6	0.147	35.1	35.4	34.8	0.796

Data for fasting insulin, 2-h postload insulin, and triglycerides were back transformed from log transformation. Data are for nondiabetic subjects only.

mographic, anthropometric, and metabolic characteristics are significantly different between the two ethnic groups. On average, the group of Mexican-Americans has fewer men, is younger, and has lower frequencies of apoE 3/2 and apoE 4/3 and a higher frequency of apoE 3/3, higher BMIs, higher subscapular-to-triceps skinfold ratios, and higher values of serum insulin and plasma glucose than the non-Hispanic white group.

Given the differences in phenotypic frequencies between the two ethnic groups, the associations between the apoE phenotypes and the relevant variables were analyzed separately for Mexican-Americans and non-Hispanic whites. The sexes were pooled and comparisons were made after adjusting for age and sex using ANCOVA. Among the anthropometric variables, only BMI (in kg/m^2) showed differences across the three apoE phenotypes. In both ethnic groups, subjects with the apoE 3/2 phenotype showed the highest average BMI (34.2 in

Mexican-Americans and 29.0 in non-Hispanic whites) and subjects with the apoE 4/3 showed the lowest average BMI (28.3 in Mexican-Americans and 26.6 in non-Hispanic whites). These BMI differences across apoE phenotypes were statistically significant in Mexican-Americans ($P = 0.044$); hence, the analyses that follow were made after further adjustment for BMI.

Table 3 shows the insulin, glucose, and lipid variables compared across apoE phenotypes after adjusting for age, sex, and BMI separately in Mexican-Americans and non-Hispanic whites. In Mexican-Americans, both fasting and 2-h postload insulin levels are significantly lower in subjects with the apoE 3/2 phenotype than in subjects with the apoE 4/3 phenotype. In non-Hispanic whites, fasting plasma glucose is significantly lower in subjects with the apoE 3/2 phenotype than in subjects with the apoE 4/3 phenotype. Skinfolds, circumferences, WHRs,

and STRs did not differ significantly by apoE phenotype.

Mexican-American subjects with the apoE 3/2 phenotype had the lowest triglyceride concentration, and those with the apoE 4/3 phenotype had the highest concentration. In non-Hispanic whites, the lowest triglyceride concentration was seen in those with the apoE 3/3 phenotype. HDL cholesterol did not show significant differences across apoE phenotypes in either ethnic group, but it tended to be higher in the apoE 3/2 carriers compared with the other two phenotypes.

Total cholesterol was lower in subjects with the apoE 3/2 phenotype than in subjects with the apoE 4/3 phenotype, but in neither ethnic group did these differences reach statistical significance. As for LDL cholesterol, subjects with the apoE 3/2 phenotype also showed the lowest levels, and those with the apoE 4/3 phenotype showed the highest levels. In Mexican-Americans the difference was statistically significant, but in non-His-

Table 4—Mean values of metabolic characteristics by sex and apoE phenotype adjusted for age, ethnicity, and BMI

	Men				Women			
	ApoE phenotype			P value	ApoE phenotype			P value
	3/2	3/3	4/3		3/2	3/3	4/3	
n	15	95	23	—	15	156	25	—
Circumferences (mm)								
Waist	996.4	989.9	1004.6	0.659	924.6	978.6	972.2	0.355
Hips	1036.3	1024.7	1025.9	0.660	1019.3	1087.0	1083.4	0.120
Skinfolds (mm)								
Subscapular	25.6	24.0	24.8	0.565	24.0	28.4	26.2	0.051
Triceps	16.5	15.3	14.8	0.652	27.6	27.7	26.1	0.626
WHR	0.959	0.965	0.974	0.718	0.884	0.898	0.893	0.842
STR	1.57	1.70	1.84	0.406	0.94	1.05	1.00	0.316
Fasting insulin (μ U/ml)	6.02	8.27	10.82	0.296	4.32	8.69	7.24	0.036
2-h insulin (μ U/ml)	31.53	43.51	67.76	0.050	23.17	58.21	70.04	0.004
Fasting glucose (mg/dl)	91.64	91.80	91.90	0.998	84.55	89.47	89.30	0.167
2-h glucose (mg/dl)	106.58	106.55	114.39	0.575	106.83	122.34	125.76	0.173
Triglycerides (mg/dl)	146.64	139.63	170.72	0.278	111.39	139.77	170.03	0.041
HDL cholesterol (mg/dl)	44.84	39.91	36.31	0.078	47.49	46.38	46.25	0.927
Total cholesterol (mg/dl)	199.10	220.61	229.99	0.218	203.53	223.59	228.29	0.139
LDL cholesterol (mg/dl)	116.00	149.83	158.36	0.022	129.05	145.06	140.53	0.329

Data for fasting insulin, 2-h postload insulin, and triglycerides were back transformed from natural logarithms. Data are for nondiabetic subjects only.

panic whites the *P* value was statistically borderline. Also included in Table 3 is the percentage of skin reflectance as an indicator of genetic admixture. Mexican-Americans with the apoE 3/2 phenotype tend to be darker (less skin reflectance) than Mexican-Americans with the other two phenotypes; however, the difference is not statistically significant. Among non-Hispanic whites, the three phenotypes show similar values of skin reflectance.

Table 4 shows the insulin, glucose, and lipid variables separately in each sex compared across apoE phenotypes after adjusting for age, ethnicity, and BMI. Fasting and 2-h insulin concentrations were lower in subjects with phenotype apoE 3/2 than in the other apoE phenotypes, although this relationship did not reach statistical significance for fasting insulin in men. Skinfolds, circumferences, WHRs, STRs, and glucose concentrations did not differ significantly by apoE phenotypes. LDL and total cholesterol concentrations were lower in subjects with apoE 3/2 in men, although the

relationship was significant only for LDL cholesterol. In women, LDL cholesterol was significantly lower in subjects with apoE 3/2.

Since all trends were similar in both ethnic groups and in both sexes, these differences were re-examined after combining the two ethnic groups and sexes, to gain more statistical power, and adjusting for ethnicity and sex (Table 5). Compared with carriers of the other two phenotypes, carriers of the apoE 3/2 phenotype showed significantly lower levels of both fasting and 2-h postload insulin. On the other hand, the apoE phenotypes did not show significant associations with circumferences, skinfolds, WHRs, STRs, or glucose measurements. Among the lipid measurements, triglyceride, LDL, and total cholesterol levels were lower in subjects with the apoE 3/2 phenotype than in subjects with the other two phenotypes, but only the differences in triglyceride and LDL levels reached statistical significance. The significance of the difference in cholesterol levels was bor-

derline. Again, HDL did not show association with the apoE phenotypes in this pooled sample.

Table 6 shows the association between insulin and lipids and lipoproteins separately in each apoE isoform. Since Després et al. (10) have suggested that the association between insulin and lipids and lipoproteins may be altered in subjects with different apoE phenotypes, we examined the associations of fasting glucose and insulin with obesity, body fat distribution, and lipids and lipoproteins in the different apoE phenotypes. We did not see consistent differences in the association between insulin or glucose and the other variables in the different apoE phenotypes. We also fit interaction terms between insulin \times apoE phenotypes and glucose \times apoE phenotypes with lipids and lipoproteins as dependent variables; these interaction terms were not statistically significant (data not shown).

Since apoE phenotype was significantly related to insulin concentrations (Table 5) and since apoE phenotype dis-

Table 5—Mean values of metabolic variables by apoE phenotype adjusted for age, sex, ethnicity, and BMI in nondiabetic subjects

	ApoE phenotype			P value
	3/2	3/3	4/3	
n	30	244	46	—
Circumferences (mm)				
Waist	954.3	983.4	989.4	0.369
Hip	1024.6	1062.6	1062.2	0.130
Skinfolds (mm)				
Subscapular	25.8	26.6	25.5	0.533
Triceps	23.0	22.6	22.7	0.595
WHR	0.918	0.925	0.928	0.845
STR	1.21	1.32	1.35	0.419
Fasting insulin (μ U/ml)	5.1	8.6	8.0	0.035
2-h postload insulin (μ U/ml)	30.1	51.6	67.9	0.003
Fasting glucose (mg/dl)	87.6	90.6	90.9	0.276
2-h postload glucose (mg/dl)	107.6	116.0	121.3	0.195
Triglycerides (mg/dl)	128.3	140.3	169.4	0.042
HDL cholesterol (mg/dl)	46.6	43.7	42.0	0.291
Total cholesterol (mg/dl)	204.3	222.7	227.7	0.064
LDL cholesterol (mg/dl)	124.7	147.3	147.1	0.022

Data for fasting insulin, 2-h postload insulin, and triglycerides were back transformed from log transformation.

tribution differed significantly by ethnic group (Table 1), we examined whether ethnic differences in apoE phenotype may explain the previously reported ethnic difference in hyperinsulinemia (14). Table 7 shows the results of multiple adjustments on the ethnic difference in hyperinsulinemia. Further adjustment for apoE slightly reduces the ethnic difference in hyperinsulinemia; however, these ethnic

differences in hyperinsulinemia remain highly significant ($P < 0.001$). In these analyses, apoE phenotype remained significantly associated with both fasting and 2-h insulin concentrations ($P < 0.05$).

CONCLUSIONS— The apoE isoform frequencies that we report here for Mexican-Americans and non-Hispanic

whites are similar to frequencies reported for other populations, including those of comparable ethnic backgrounds (4,24–27). Also, we have verified the amply documented association of the apoE2 and the apoE4 isoforms with, respectively, low and high levels of LDL cholesterol. This association was similar in both ethnic groups, but it was statistically significant only in Mexican-Americans, probably be-

Table 6—Pearson's partial correlation coefficients of fasting glucose and insulin with lipid and anthropometric variables by apoE phenotype adjusted for sex and ethnicity

	Fasting insulin						Fasting glucose					
	ApoE 3/2		ApoE 3/3		ApoE 4/3		ApoE 3/2		ApoE 3/3		ApoE 4/3	
	CC	P value	CC	P value	CC	P value	CC	P value	CC	P value	CC	P value
BMI	0.065	0.756	0.277	<0.001	0.422	0.049	0.293	0.155	0.199	0.002	0.286	0.067
WHR	0.234	0.261	0.088	0.173	0.063	0.686	0.547	0.005	0.203	0.002	0.372	0.014
STR	0.328	0.110	0.069	0.287	0.189	0.224	0.500	0.011	0.086	0.183	0.290	0.059
Tg	0.106	0.615	0.134	0.038	0.107	0.496	−0.091	0.664	0.220	0.001	0.174	0.265
HDL	−0.157	0.454	−0.090	0.164	−0.342	0.025	0.020	0.925	−0.185	0.004	−0.891	0.570
TC	−0.314	0.126	0.020	0.750	−0.084	0.594	−0.249	0.230	0.080	0.214	0.118	0.451
LDL	−0.466	0.019	0.005	0.935	−0.081	0.605	−0.254	0.220	0.044	0.499	0.077	0.619

CC, Pearson's correlation coefficient; Tg, triglycerides; TC, total cholesterol. For ApoE 3/2, 3/3, and 4/3, $n = 27$, 242, and 45, respectively.

Table 7—Insulin concentrations in nondiabetic Mexican-Americans and non-Hispanic whites

	Mexican-Americans	Non-Hispanic whites	Difference	P value
Adjusted for age, sex				
Fasting insulin	14.3 ± 1.2	10.2 ± 1.96	4.1	<0.001
2-h insulin	96.9 ± 5.8	56.4 ± 7.7	40.5	<0.001
Adjusted for age, sex, BMI, WHR				
Fasting insulin	13.9 ± 1.2	10.7 ± 1.6	3.2	<0.001
2-h insulin	94.0 ± 5.7	61.9 ± 7.6	32.1	<0.001
Adjusted for age, sex, BMI, WHR, fasting glucose				
Fasting insulin	13.9 ± 1.2	10.8 ± 1.6	3.1	<0.001
2-h insulin	93.3 ± 5.7	63.2 ± 7.6	30.1	<0.001
Adjusted for age, sex, BMI, WHR, fasting glucose, apoE phenotype				
Fasting insulin	13.7 ± 1.7	10.8 ± 1.8	2.9	0.003
2-h insulin	94.5 ± 7.9	65.3 ± 8.4	29.2	<0.001

Data are means ± SE in $\mu\text{U/ml}$.

cause of their larger sample size. Particularly in Mexican-Americans (Table 3), subjects with the apoE 3/2 phenotype showed the lowest average level of triglycerides, in contrast with findings in other populations where subjects with the apoE 3/3 phenotype either show the lowest level of triglycerides (28) or show no difference from the other two phenotypes (10,24). We also found the effect of apoE on lipids and lipoproteins and insulin to be similar in both sexes.

The major aim of this report was to test further for associations between the apoE polymorphism and other CAD risk factors such as insulin and glucose levels in blood, which are indicators of insulin resistance. This aim is justified by observations indicating that the effect of apoE on LDL cholesterol levels may require cofactors to activate the receptor-mediated uptake of this lipoprotein, since apoE is not a structural part of LDL (29). One of these cofactors could be insulin. For example, it has been shown that the uptake of apoE-enriched lipoproteins is doubled or tripled when isolated rat adipocytes are exposed to physiological concentrations of insulin (30), and there is evidence suggesting that the apoE polymorphism may modulate the association

of insulin with lipid levels in humans (10).

We have found that compared with the other two major apoE phenotypes, the apoE 3/2 phenotype is associated with low levels of fasting and 2-h postload insulin in nondiabetic subjects. This association seems more marked in Mexican-Americans than in non-Hispanic whites; however, the ethnic difference could be due to a lack of statistical power in non-Hispanic whites, because when the ethnic groups are pooled, the association remains significant even after the effects of age, sex, BMI, and ethnicity have been accounted for. Interestingly, the association of the apoE 3/2 phenotype with low insulin levels parallels the already known association of this phenotype with low levels of total and LDL cholesterol. Our data also suggest that the association between apoE phenotype and insulin concentrations and lipids and lipoproteins was similar in the both sexes.

Després et al. (10) suggest that the relationship between insulin and triglyceride is stronger in subjects with apoE 2/2 and apoE 3/3 than in subjects with apoE 4/4. We do not understand the differences in these two studies, but they could relate to the small number of subjects in the two

studies. Alternatively, we assessed only the heterozygotes apoE 3/2 and apoE 4/2, while Després et al. (10) assessed the homozygotes apoE 2/2 and apoE 4/4. We performed analyses analogous to Table 6 separately in Mexican-Americans and non-Hispanics but did not see a differential effect in either Mexican-Americans or non-Hispanic whites. Thus, we do not believe that the differences in the two reports are a result of ethnic differences. A last possible cause is that the Canadian study (10) included only premenopausal women and our women were predominantly postmenopausal.

We also considered whether adjustment for apoE phenotype might explain the previously reported ethnic differences in hyperinsulinemia (14), since apoE phenotype distribution was significantly different in Mexican-Americans and non-Hispanic whites (Table 2) and apoE phenotype was related to insulin concentrations (Table 5). Although apoE phenotype slightly reduced the ethnic differences in insulin concentrations, these differences remained highly significant (Table 7). We conclude that apoE does not make a major contribution to higher insulin levels seen in Mexican-Americans.

In two other studies that have included Hispanic subjects, plasma insulin concentrations among nondiabetic subjects have been compared across the three major apoE phenotypes, but no significant associations have been found (10,24). We cannot fully explain this discrepancy with our results, except to say that our study population may have included an abundance of hyperinsulinemic subjects (14). Therefore, although restricted to nondiabetic subjects, our study included a wide range of insulin values, which in turn may have permitted us a better discrimination across the apoE phenotypes. An alternative explanation is that since the isoform apoE2 is more frequent among Caucasians, the lower values of fasting insulin in Mexican-Americans with the phenotype apoE 3/2 may be indicative of a higher proportion of Caucasian admixture in this group. Skin color, however, was actually darker in Mexican-Americans with the apoE 3/2 phenotype (although not statistically significantly so) suggesting that if anything they had less Caucasian admixture. Moreover, studies in other Hispanic populations with noticeable Caucasian admixture have found no association of apoE phenotypes with fasting insulin values.

The implication of our study is that the intra- and interpopulation variations introduced by the apoE phenotypes in lipid regulation may be extended to insulin-related conditions as well, which could make this polymorphism part of a much broader set of conditions that ultimately lead to cardiovascular disease. However, given the small sample size of some of our major phenotype groups, our results have to be interpreted with caution and need validation in a larger population.

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