

Obesity Modulates the Association among *APOE* Genotype, Insulin, and Glucose in Men

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Abstract

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Objective: Obesity, insulin resistance, and apolipoprotein E (*APOE*) genotype have all been associated with coronary heart disease. We examined the interaction between obesity and *APOE* genotype in determining fasting insulin and glucose levels.

Research Methods and Procedures: From 1991 to 1995, 3799 subjects underwent a clinical examination and fasting insulin and glucose measurement. *APOE* genotypes were determined on 3500 participants. Participants taking oral hypoglycemic drugs or insulin preparations or with the rare *APOE2/4* genotype were excluded. Finally, 2929 individuals were included in the present analysis.

Results: In men, we observed a statistically significant interaction between obesity and *APOE* genotype on insulin and glucose level ($p = 0.003$ and 0.008 , respectively). Obese men with the *APOE4* genotype presented with higher levels of insulin and glucose than obese men in the other genotype groups. No association between genotype and insulin or glucose in nonobese men was observed. Obesity was associated with higher insulin levels in the three *APOE* genotypes groups, whereas obesity was directly associated with glucose in those with the *APOE4* genotype. In women,

the effect of interaction between *APOE* genotype and obesity on fasting insulin and glucose was not statistically significant. Obesity was associated with higher levels of fasting insulin and glucose. *APOE* genotype was not associated with insulin or glucose.

Discussion: Obesity modulates the association between the *APOE* genotype and fasting insulin and glucose levels in men. Although weight control is important in all people, it may be especially important in *APOE4* men to modify potentially elevated fasting insulin and glucose levels.

Key words: *APOE* genotype, insulin, glucose, interaction

Introduction

Hyperinsulinemia has been associated independently with a higher risk of incident coronary heart disease (CHD)¹ (1–3), primarily in men (4,5). However, a direct relationship between insulin and CHD remains controversial because effects of insulin may be mediated by correlated risk factors, other comorbidities, and genetic factors (6,7).

Apolipoprotein E (apoE) is a plasma protein modulating metabolism of plasmatic lipoproteins, particularly apoB-containing lipoproteins. The *APOE* locus is polymorphic, with three major alleles ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) encoding the six most common isoforms. This genetic variation significantly affects plasma lipoproteins concentrations. The presence of the $\epsilon 4$ allele is associated with elevated low-density lipoprotein (LDL) cholesterol, whereas the presence of the $\epsilon 2$ allele is associated with decreased LDL cholesterol (LDL-C) (8). Moreover, $\epsilon 2$ and $\epsilon 4$ have also been found to be associated with elevations in plasma triglycerides (9) and/or higher cardiovascular disease risk (8,10,11). Conversely, the association between *APOE* genotype and insulin resistance and diabetes has been addressed in several studies, including our own (12), with negative results (13,14). The aim of the

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¹ Nonstandard abbreviations: CHD, coronary heart disease; apo, apolipoprotein; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; CVD, cardiovascular disease.

current study was to determine whether the association between APOE genetic variation and fasting insulin and glucose levels is modulated by obesity.

Research Methods and Procedures

Study Population

Participants in the Framingham Offspring Study, a long-term community-based prospective observational study of risk factors for cardiovascular diseases, were included in this study (15). From January 1991 through June 1995 (examination cycle 5), 3799 participants fasted overnight; each had standardized medical history and physical and laboratory examination. From these, 3500 participants had the APOE genotype determined. For these analyses, 118 participants were excluded for taking oral hypoglycemic drugs or insulin preparations, and 404 were excluded because no valid information was collected for variables of interest. We also excluded from the analysis 49 individuals with the rare APOE2/4 genotype, leaving 2929 individuals in the present analysis.

Laboratory Methods: Fasting Glucose and Insulin

Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (A-gen glucose test; Abbot, South Pasadena, CA). Glucose assays were run in duplicate; the intra-assay coefficient of variation was <3%. Fasting insulin was measured in EDTA plasma as total immunoreactive insulin (Coast-A-Court Insulin; Diagnostic Products, Los Angeles, CA) and calibrated to serum levels for reporting purposes. Cross-reactivity of this assay with pro-insulin at mid-curve was ~40%, the intra- and interassay coefficient of variation ranged from 5.0 to 10.0%, and the lower limit of sensitivity was 8 pM.

Laboratory Methods: APOE Genotype

Leukocyte DNA was extracted from 5 to 10 mL of whole blood, as previously described (16) and according to current guidelines (17). APOE genotyping was performed as described by Hixson and Vernier (18). A 244-bp sequence of the APOE gene including the two polymorphic sites was amplified by polymerase chain reaction in a DNA Thermal Cycler (PTC-100; MJ Research, Watertown, MA), using oligonucleotide primers F4 and F6 (18). Each reaction mixture was heated at 94 °C for 2 min, followed by 35 cycles of amplification (94 °C for 40 s, 62 °C for 30 s, and 72 °C for 1 min). The polymerase chain reaction products were digested with 5 U of Hha I, and the fragments were separated by electrophoresis on an 8% polyacrylamide non-denaturing gel. After electrophoresis, the gel was treated with ethidium bromide for 30 min, and DNA fragments were visualized by UV illumination.

Three APOE genotypes were defined: APOE2 for those subjects carrying the $\epsilon 2/\epsilon 2$ or $\epsilon 2/\epsilon 3$ genotypes, APOE3 for

those carrying the $\epsilon 3/\epsilon 3$ genotype, and APOE4 for those carrying the $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ genotypes.

Other Variables

Height and weight were measured with the individual dressed in an examining gown and wearing no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters (kilograms per meter squared). Obesity was defined as BMI ≥ 30 kg/m². Waist circumference was determined, and abdominal obesity was defined as a waist circumference higher than 102 cm in men and higher than 88 cm in women. Smoking status was based on the cigarette consumption in the year before the examination. Alcohol consumption and the use of beta-blockers, diuretics, and hormonal substitutive treatment (in women) were assessed by questionnaire. Dietary intake was estimated with the semiquantitative Willett food frequency questionnaire (19).

Ethical Issues

The study was approved by all the institutional review committees. All the subjects gave informed consent.

Statistical Analysis

χ^2 tests to compare proportions across groups and ANOVA to compare means of continuous variables across groups were used. Analysis of covariance was used to evaluate interactions between APOE genotypes and obesity in determining fasting insulin and glucose. In this analysis, we adjusted for potential confounding variables and accounted for correlations due to familial relationships among the members of the study using Proc Mixed in SAS (SAS Institute Inc., Cary, NC). Scheffe adjustment was used to control for multiple comparisons. A two-tailed *p* value < 0.05 was considered as statistically significant. The SAS program was used for statistical analysis.

Results

Characteristics of the study population are presented in Table 1. Framingham Offspring Study participants are primarily of mixed European white ethnicity. The relative frequencies of APOE genotypes are shown in Table 1. No significant differences in genotype or allele frequencies between men and women were observed.

The characteristics of participants according to APOE genotype and stratified by sex are presented in Table 2. An association between APOE genotype and insulin levels was observed in men but not in women. No other statistically significant differences were observed.

In men, the effects of interactions between APOE genotype and obesity on fasting insulin and glucose concentrations were statistically significant (*p* = 0.003 and 0.008, respectively). Among obese men, glucose was marginally significantly higher, and insulin was significantly higher in those

Table 1. Study population characteristics

	Men (n = 1410)	Women (n = 1519)
Age (years)*	55.2 (10.1)	54.9 (9.9)
APOE genotypes (%)		
$\epsilon 2/\epsilon 2$	0.5	0.2
$\epsilon 2/\epsilon 3$	12.4	14.2
$\epsilon 3/\epsilon 3$	66.9	64.5
$\epsilon 3/\epsilon 4$	18.2	19.8
$\epsilon 4/\epsilon 4$	2.1	1.4
BMI (kg/m ²)*	28.2 (4.1)	26.7 (5.5)
Obesity (%)	26.6	21.7
Waist circumference (cm)*	99.1 (10.7)	87.1 (14.5)
Abdominal obesity (%)	33.4	38.2
Alcohol consumers (%)	74.9	64.8
% Energy from saturated fat*	10.6 (2.9)	10.4 (2.9)
Smoking (%)	19.2	19.5
Beta-blockers (%)	11.7	9.0
Diuretics (%)	5.6	8.6
Hormonal substitutive treatment (%)		18.5
Fasting insulinemia (pM)	31.8 (13.4)	28.5 (10.7)
Fasting glucose (mg/dL)*	101.5 (24.4)	95.4 (17.4)

* Mean (SD).

with the *APOE4* genotype compared with those in the *APOE3* genotype group ($p = 0.057$ and 0.022) (Table 3). No association between *APOE* genotype and insulin or glucose in nonobese men was observed. Obesity was associated with higher fasting insulin in the three genotype groups and was associated with higher fasting glucose only in *APOE4* men.

In contrast, we did not find similar patterns in women, where the interaction between *APOE* genotype and obesity on fasting insulin and glucose was not statistically significant. Obesity was associated with higher levels of fasting insulin and glucose independently of the *APOE* genotype. No association between the *APOE* genotype and insulin or glucose was observed.

Moreover, we examined the interaction between abdominal obesity and *APOE* genotype on insulin and glucose levels (Table 4). The data show similar trends to those

observed for obesity. However, the interaction terms did not reach statistical significance at the level of $p < 0.05$.

A similar analysis was performed considering diabetes as the dependent variable. The 118 participants under hypoglycemic treatment (excluded for the previous analyses) and those with a fasting glucose level ≥ 126 mg/dL were considered diabetics. In men, the interaction between *APOE* genotype and obesity on diabetes was not statistically significant, although the p value was 0.069. The prevalence of diabetes among *APOE2*, *APOE3*, and *APOE4* genotype groups was 13.8%, 14.4%, and 25.0%, respectively. In women, this interaction was not statistically significant ($p = 0.999$). Similar results were obtained when the cut-off point to define diabetes was 140 instead of 126 mg/dL.

Discussion

Our previous analysis on this population did not reveal significant associations between variability at the *APOE* locus and insulin resistance (12). In view of the potential interactions between this locus and several environmental factors (20), we focused on the current analyses of interaction models, and we found statistically significant interactions between *APOE* genotype and obesity on insulin and glucose levels in men. Thus, male subjects carrying the $\epsilon 4$ allele had significantly higher fasting insulin and glucose concentrations only in the presence of obesity (BMI ≥ 30 kg/m²).

Our findings are in partial agreement with those reported by Kataoka et al. (21) in Native Americans. In this population, with a high prevalence of diabetes and obesity, these authors observed higher levels of fasting insulin in nondiabetic women with the *APOE3* and *APOE4* genotype compared with those with the genotype $\epsilon 2$ ($p = 0.03$); in men, the same tendency was observed, although without reaching statistical significance. Valdez et al. (22) also observed that the *APOE3* and *APOE4* genotypes were associated with higher levels of fasting insulin and LDL-C in two different ethnic groups (Mexican Americans and non-Hispanic whites from San Antonio) where the prevalence of obesity is higher. This association has not been observed in other studies (13,14). However, those studies did not examine the interaction between *APOE* genotype and obesity.

At this time, there are no clearly defined mechanisms to explain the modulation of the association between *APOE* genotype and insulin by obesity. One potential link has been established between lipid peroxidation and insulin resistance (23–25), suggesting that lipid peroxidation precedes insulin resistance (23,25). Increased total and LDL-C (8,26) and decreased LDL diameter (27–29) may accelerate lipid peroxidation and are associated with the *APOE4* genotype and obesity. A speculative mechanism is that the coexistence of these environmental and genetic factors could promote lipid peroxidation leading to higher insulin level.

Table 2. Characteristics of the population and insulin and glucose levels according to APOE genotype and stratified by sex

	Men				Women			
	APOE2‡ (n = 182)	APOE3‡ (n = 943)	APOE4‡ (n = 285)	p	APOE2‡ (n = 219)	APOE3‡ (n = 979)	APOE4‡ (n = 321)	p
Age (years)*	55.4 (10.0)	55.1 (10.1)	55.4 (10.1)	0.900	55.6 (9.4)	54.9 (10.1)	54.5 (9.7)	0.443
BMI (kg/m ²)*	28.3 (4.0)	28.2 (4.1)	28.0 (3.8)	0.624	26.7 (5.5)	26.6 (5.4)	27.1 (5.8)	0.397
Obesity (%)	30.4	26.3	25.3	0.440	21.1	21.3	23.5	0.680
Waist circumference (cm)*	100.1 (10.9)	99.1 (10.9)	98.6 (10.4)	0.289	87.4 (14.2)	86.9 (14.2)	88.1 (15.0)	0.391
Abdominal obesity (%)	37.9	32.3	34.0	0.324	41.7	37.0	39.5	0.371
Smoking (%)	22.0	18.8	19.0	0.601	19.3	20.1	17.8	0.647
Alcohol (%)	79.1	75.0	71.6	0.183	66.2	63.8	67.2	0.485
% Energy from SF*†	10.9 (2.8)	10.7 (3.0)	10.2 (2.7)	0.059	10.4 (2.7)	10.4 (2.9)	10.4 (2.8)	0.888
Beta-blockers (%)	13.2	11.1	12.6	0.631	8.7	9.1	9.0	0.980
Diuretics (%)	7.1	5.5	4.9	0.581	8.7	8.5	8.7	0.990
Insulin (pM)*	31.7 (12.1)	31.2 (12.3)	33.7 (16.9)	0.031	29.4 (13.6)	28.1 (10.1)	28.9 (10.4)	0.219
Glucose (mg/dL)*	103.0 (27.3)	100.7 (22.8)	103.5 (27.2)	0.152	95.0 (15.3)	95.1 (15.4)	96.6 (23.4)	0.377

* Mean (SD).

† SF, saturated fat.

‡ APOE2 group includes ε2/ε2 and ε2/ε3 genotypes; APOE3 group includes ε3/ε3; and APOE4 group includes ε3/ε4 and ε4/ε4 genotypes.

Table 3. Fasting insulin and glucose distribution [mean (SE)] according to APOE genotype and obesity in all the population and stratified by sex

	Fasting insulin (pM)			Fasting glucose (mg/dL)		
	BMI < 30 kg/m ²	BMI ≥ 30 kg/m ²	p ₁ †	BMI < 30 kg/m ²	BMI ≥ 30 kg/m ²	p ₁ †
Men						
APOE2	28.78 (1.13)	37.74 (1.75)	0.003	100.77 (2.06)	103.25 (3.18)	0.994
APOE3	28.27 (0.48)	39.93 (0.82)	<0.001	99.50 (0.88)	104.65 (1.50)	0.123
APOE4	28.72 (0.88)	46.26 (1.52)	<0.001	99.50 (1.60)	115.06 (2.78)	<0.001
p ₂ †	NS	E2 vs E4: 0.020 E3 vs E4: 0.022	p ₃ † = 0.003	NS	E3 vs E4: 0.057	p ₃ = 0.008
Women						
APOE2	27.93 (0.75)	36.55 (1.62)	<0.001	92.89 (1.30)	103.53 (2.66)	0.026
APOE3	26.04 (0.37)	35.75 (0.72)	<0.001	92.81 (0.63)	103.27 (1.22)	<0.001
APOE4	26.40 (0.64)	36.40 (1.18)	<0.001	93.49 (1.10)	107.33 (2.02)	<0.001
p ₂ †	NS	NS	p ₃ † = 0.816	NS	NS	p ₃ † = 0.439

Adjusted for age, smoking, alcohol, saturated fat intake, and beta-blocker and diuretic use (in women also by hormonal substitutive treatment).

* APOE2 group includes ε2/ε2 and ε2/ε3 genotypes; APOE3 group includes ε3/ε3; APOE4 group includes ε3/ε4 and ε4/ε4 genotypes.

† p₁ = p value for obesity; p₂ = p value for APOE genotype; p₃ = p value for the interaction obesity-APOE genotype.

Table 4. Fasting insulin and glucose distribution [mean (SE)] according to APOE genotype and abdominal obesity in all the population and stratified by sex

	Fasting insulin (pM)			Fasting glucose (mg/dL)		
	Waist circumference ≤ 102 cm/ ≤ 88 cm	Waist circumference > 102 cm/ > 88 cm	p_1^\dagger	Waist circumference ≤ 102 cm/ ≤ 88 cm	Waist circumference > 102 cm/ > 88 cm	p_1^\dagger
Men	≤ 102 cm	> 102 cm		≤ 102 cm	> 102 cm	
APOE2	27.56 (1.81)	38.60 (1.59)	< 0.001	101.38 (2.21)	103.23 (2.92)	0.998
APOE3	27.75 (0.50)	38.92 (0.74)	< 0.001	99.74 (0.95)	103.20 (1.38)	0.504
APOE4	28.42 (0.95)	41.81 (1.29)	< 0.001	99.91 (1.76)	109.36 (2.40)	0.072
p_2^\dagger	NS	NS	$p_3^\dagger = 0.458$	NS	NS	$p_3^\dagger = 0.152$
Women	≤ 88 cm	> 88 cm		≤ 88 cm	> 88 cm	
APOE2	27.26 (0.89)	32.94 (1.10)	0.007	92.67 (1.51)	98.22 (1.84)	0.363
APOE3	25.33 (0.42)	32.78 (0.55)	< 0.001	91.86 (0.71)	100.28 (0.93)	< 0.001
APOE4	25.16 (0.72)	34.44 (0.93)	< 0.001	92.02 (1.22)	104.12 (1.58)	< 0.001
p_2^\dagger	NS	NS	$p_3^\dagger = 0.140$	NS	NS	$p_3^\dagger = 0.094$

Adjusted for age, smoking, alcohol, saturated fat intake, and beta-blocker and diuretic use (in women also by hormonal substitutive treatment).

* APOE2 group includes $\epsilon 2/\epsilon 2$ and $\epsilon 2/\epsilon 3$ genotypes; APOE3 group includes $\epsilon 3/\epsilon 3$; APOE4 group includes $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ genotypes.

$^\dagger p_1 = p$ value for obesity; $p_2 = p$ value for APOE genotype; $p_3 = p$ value for the interaction obesity-APOE genotype.

Alternatively, obesity and APOE4 are also associated with higher triglyceride concentrations (9), and interactions between obesity and APOE4 on plasma triglycerides have been reported (30). Patients at an early stage of insulin resistance already have lipoprotein alterations characterized by increased very-low-density lipoprotein, intermediate-density lipoprotein, and LDL apoB production rates (31) and show a lower lipolytic capacity (32). The interaction between APOE and obesity was observed in men but not in women. Notably, the association between the APOE4 genotype and cardiovascular disease (CVD) or CHD has been mainly reported in men but less consistently in women (8,33). In particular, the presence of allele $\epsilon 4$ (8,10,11) has been associated with higher CVD risk; in the present study, the $\epsilon 4$ allele was associated with higher insulin and glucose levels in obese men. In addition, hyperinsulinemia has been associated with excess CVD risk in men but not in women (34). Moreover, the excess of CVD risk observed in men with the APOE4 has been confined to hyperinsulinemic men in the presence of glucose intolerance, obesity, or hypertension (34).

To our knowledge, the possible modulating effect of obesity on the relationship between APOE genotype or insulin and cardiovascular risk has not been documented previously. The present data seem to represent an obesity-genotype interaction on CVD risk factors and suggest the

hypothesis that obesity and APOE genotype may interact in men to intensify insulin resistance or hyperinsulinemia, leading to increased CVD risk. Among women, the better lipid profile, with a higher concentration of HDL and greater size of LDL particles (35), may protect them from the deleterious combination of APOE4 and obesity on insulin levels.

The interaction between abdominal obesity and APOE genotype on insulin or glucose levels was not statistically significant in men or in women. These results suggest that, at least in this population, global obesity (defined as BMI > 30 kg/m²) is a more sensitive variable than abdominal obesity for the detection of the interaction between APOE genotype and obesity on insulin and glucose levels.

In conclusion, obesity modulates the association between APOE genotype and fasting insulin and glucose levels in men. Obese men with the $\epsilon 4$ genotype are more hyperinsulinemic and hyperglycemic than obese men with the $\epsilon 2$ or $\epsilon 3$ genotypes, whereas nonobese men have lower glucose and insulin levels than obese men but have similar glucose and insulin levels across APOE genotypes. These data seem to represent a novel phenotype (obesity)-genotype interaction that affects CVD risk factor levels. Although weight control is important in all people to improve insulin sensitivity and reduce CVD risk, weight control may be espe-

cially important in *APOE4* subjects to modify potentially elevated fasting insulin and glucose levels and lower consequent CVD risk.

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