

Influence of Apolipoproteins on the Association Between Lipids and Insulin Sensitivity

A cross-sectional analysis of the RISC Study

SIMONA BALDI, PHD¹
 FABRICE BONNET, MD²
 MARTINE LAVILLE, MD³
 CECILIA MORGANTINI, MD⁴
 LUCILLA MONTI, MD⁵

KURT HOJLUND, MD⁶
 ELE FERRANNINI, MD¹
 ANDREA NATALI, MD¹
 ON BEHALF OF THE RISC INVESTIGATORS*

OBJECTIVE—We evaluated whether the association of insulin sensitivity with HDL cholesterol (HDL) and triglycerides is influenced by major plasma apolipoproteins, as suggested by recent experimental evidence.

RESEARCH DESIGN AND METHODS—This study included a cross-sectional analysis of the RISC Study, a multicenter European clinical investigation in 1,017 healthy volunteers balanced in sex (women 54%) and age strata (range 30–60 years). Insulin sensitivity (M/I in $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}_{\text{FFM}}^{-1} \cdot \text{nM}^{-1}$) was measured by the clamp technique and apolipoproteins (ApoB, -C3, -A1, and -E) by Multiplex Technology.

RESULTS—The center-, sex-, and age-adjusted standardized regression coefficients (STDB) with M/I were similar for HDL and triglycerides ($+19.9 \pm 1.9$ vs. -20.0 ± 2.0 , $P < 0.0001$). Further adjustment for triglycerides (or HDL), BMI, and adiponectin (or nonesterified fatty acid) attenuated the strength of the association of M/I with both HDL (STDB $+6.4 \pm 2.3$, $P < 0.01$) and triglycerides (-9.5 ± 2.1 , $P < 0.001$). Neither ApoA1 nor ApoE and ApoB showed any association with M/I independent from plasma HDL cholesterol and triglycerides. ApoC3, in contrast, in both men and women, was positively associated with M/I independently of plasma lipids. A relative enrichment of plasma lipids with ApoC3 is associated with lower body fat percentage and lower plasma alanine amino transferase.

CONCLUSIONS—Our results suggest that HDL cholesterol modulates insulin sensitivity through a mechanism that is partially mediated by BMI and adiponectin but not by ApoA1. Similarly, the influence of triglycerides on insulin sensitivity is in part mediated by BMI and is unrelated to ApoE or ApoB, but it is significantly modulated by ApoC3, which appears to protect from the negative effect of plasma lipids.

Diabetes Care 36:4125–4131, 2013

The dyslipidemia characterized by elevated plasma triglycerides and low HDL cholesterol is one of the key components of the metabolic syndrome, and there is evidence that it is

secondary to the presence of insulin resistance and obesity (1). However, recent data suggest that serum lipoproteins exert an influence on tissue insulin sensitivity both directly and through the activity of

their major lipoproteins. HDL particles and apolipoprotein A-I (ApoA1) might indirectly modulate insulin sensitivity through their antioxidant and anti-inflammatory action (2), their effect on energy expenditure (3), and/or their close relationships with adiponectin (4,5). Apolipoproteins CIII (ApoC3) and E (ApoE), which are present both in VLDL and HDL particles, have the potential to modulate whole-body insulin sensitivity by regulating the transfer of plasma triglycerides and nonesterified fatty acids (FFAs) to tissues. ApoC3, the second most abundant apolipoprotein circulating in human plasma, is a natural lipoprotein lipase inhibitor (6–8). When exposed to a high-fat diet, *APOC3* gene knockout mice display lower serum triglycerides but more severe obesity and insulin resistance, which are caused by excess FFA transfer from plasma to tissues (9).

Data in humans are scarce and conflicting. Higher ApoC3 levels have been reported in subjects with increased triglycerides, insulin resistance, cardiovascular disease, and type 2 diabetes (10–12). However, the contribution of ApoC3, independent of triglycerides, remains unclear. Although studies on the human *APOC3* gene have failed to show clear associations between mutations and altered lipid/lipoprotein metabolism (7), interestingly, *APOC3* polymorphisms rs2854116 and rs2854117 are associated with a relative poverty of ApoC3 (with respect to serum triglycerides), increased liver triglyceride content, and insulin resistance (13). Similarly, in middle-aged men with the metabolic syndrome, lower ApoC3 levels were associated with a higher visceral fat mass and lower plasma triglycerides (14).

Data from experimental animals suggest that ApoE is opposite to ApoC3, its lack being associated with resistance to the development of both diet-induced obesity and insulin resistance (15). In humans, elevated levels of ApoE are consistently found in obesity, insulin resistance, and dyslipidemia (16), and all these factors, in turn, have been shown to upregulate ApoE

From the ¹Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; the ²Service Endocrinologie-Diabétologie, Centre Hospitalo-Universitaire Rennes, University Rennes 1, Rennes, France; the ³Centre de Recherche en Nutrition Humaine Rhône-Alpes, INSERM U870-INRA 1235, Lyon, France; the ⁴Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy; the ⁵Cardio-Diabetes and Core Laboratory Unit, Metabolic and Cardiovascular Science Division, San Raffaele Scientific Institute, Milan, Italy; and the ⁶Department of Endocrinology, Odense University Hospital, Odense, Denmark.

Corresponding author: Simona Baldi, simona.baldi@dmi.unipi.it.

Received 21 March 2013 and accepted 4 July 2013.

DOI: 10.2337/dc13-0682

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-0682/-/DC1>.

*A complete list of the RISC Study investigators can be found in the APPENDIX.

© 2013 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

and VLDL production (17). Therefore, it is particularly difficult to evaluate whether ApoE modulates insulin sensitivity independently of serum triglycerides and obesity. Also the studies on ApoE $\epsilon 2/\epsilon 3/\epsilon 4$ polymorphisms have not reached consistent conclusions. The Framingham Offspring Study found that none of these polymorphisms was associated with insulin resistance (18), whereas other studies (19–21) have observed that the polymorphisms modify the strength of the associations between insulin resistance and dyslipidemia.

In conclusion, although experimental data suggest a role for apolipoproteins in modulating insulin sensitivity, whether this holds true in humans remains unclear. Possible reasons are that in most previous studies, insulin sensitivity was not directly measured but only estimated through plasma insulin and glucose levels, and/or that the strategies to dissect the effect of the apolipoproteins from that of lipoprotein as well as from their confounders were not always appropriate.

To directly address the question of whether ApoA1, ApoC3, and ApoE modulate the relationships of serum lipids with insulin sensitivity, we exploited the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) Study (22), one of the largest collections of subjects in which insulin sensitivity was measured with the use of the gold standard technique (the euglycemic-hyperinsulinemic clamp).

RESEARCH DESIGN AND METHODS

Study participants

RISC is a prospective, observational cohort study; the rationale and methodology have been described previously (22). In brief, participants were recruited at 19 centers in 14 European countries, according to the following inclusion criteria: either sex, age 30–60 years, and clinically healthy. Exclusion criteria were as follows: treatment for any chronic disease, pregnancy, any cardiovascular event, weight change of ≥ 5 kg in last 3 months, cancer (in the last 5 years), and renal or liver failure, arterial blood pressure $\geq 140/90$ mmHg, fasting plasma glucose ≥ 7.0 mmol/L, 2-h plasma glucose (on a standard 75-g oral glucose tolerance test) ≥ 11.0 mmol/L, total serum cholesterol ≥ 7.8 mmol/L, serum triglycerides ≥ 4.6 mmol/L, or electrocardiogram abnormalities. The present analysis is based on the

baseline data of 1,017 subjects who satisfied all criteria and in whom the apolipoproteins were measured. Local ethics committee approval was obtained by each recruiting center.

Lifestyle and medical history

Information on menopause, as well as on smoking habits and alcohol consumption, was collected by the MD responsible for the study. Smoking status was categorized as current, ex, or never and only referred to cigarette smoking. Physical activity was assessed by means of the International Physical Activity Questionnaire (IPAQ) (www.ipaq.ki.se), a validated estimate for the number of metabolic equivalents per week; these estimates were compacted into a three-level score: 1, inactive; 2, minimally active; and 3, health-enhancing physical activity.

Physical examinations

Height was measured on a clinic stadiometer, and body weight, percent body fat, and fat-free mass (FFM) were evaluated by bioimpedance (Tanita International Division, Tokyo, Japan). Sitting blood pressure and heart rate were measured (OMRON 705 cp; OMRON Healthcare Europe, Hoofddorp, the Netherlands) three times over 10 min, and the median value was used in statistical analyses.

Insulin clamp

Euglycemic-hyperinsulinemic clamp was performed in all subjects. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 pmol/min/m² simultaneously with a variable 20% dextrose infusion adjusted every 5–10 min to maintain plasma glucose level within 15% of the target glucose level (4.5–5.5 mmol/L). Additional blood samples were obtained at 20-min intervals for insulin and FFA determination.

Analytical methods

Blood was separated into plasma and serum, aliquoted, and stored at -80°C for centralized analyte determination. Serum total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured by enzymatic colorimetric test (Roche Modular Systems), FFA by an immunoenzymatic assay (Randox), and plasma insulin and C-peptide by a two-sided, time-resolved fluoroimmunoassay (AutoDELFIA Insulin Kit; Wallac Oy, Turku, Finland). Serum ApoA1, -A2, -B100, -C3, and -E were assayed with Milliplex APO-62 K kits on a Bio-Plex

200 System instrument (Bio-Rad Laboratories). Serum adiponectin was measured by an in-house time-resolved immunofluorometric assay based on two antibodies and recombinant human adiponectin (R&D Systems, Abingdon, U.K.), as previously described (23).

Calculations

Insulin sensitivity (M/I, in units of $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}_{\text{FFM}}^{-1} \cdot \text{nM}^{-1}$) was expressed as the ratio of the M value, averaged over the final 40 min of the 2-h clamp and normalized by the FFM, to the mean plasma insulin concentration measured during the same interval (24).

Statistical analysis

Data are reported as mean \pm SD. Differences between means and prevalence rates have been evaluated with Student *t* test and χ^2 , respectively. General linear models (standard least square) were used to test the simultaneous dependence of continuous variables on single or multiple parameters. Since none of the lipid variables displayed a normal distribution, we preliminarily verified the consistency of the correlations by comparing Pearson *r* and Spearman ρ of the nontransformed variable with the Pearson *r* of the log transformed (Supplementary Table 1). Since all the correlation coefficients were similar, we used the standardized (values/sex-specific SD) plasma lipids and apolipoproteins to allow a direct comparison between variables that is independent of their units and their biological range. The estimated regression coefficients were therefore called standardized regression coefficients (STD β) and expressed as value \pm SEM. Two-way ANOVA and ANCOVA were used for group comparisons while controlling for covariates. A two-tailed *P* value of ≤ 0.05 was considered statistically significant. All analyses were run on JMP 9.0.1.

RESULTS—Men and women differed with respect to insulin sensitivity, anthropometric and metabolic characteristics, and lipid profile (Table 1), with women showing an overall healthier profile despite a higher fat mass and plasma FFA concentrations. ApoC3 and ApoB were lower in women than men, whereas ApoA1 was higher; ApoE did not show a sex difference. Menopause was associated with a diffuse (10–15%) increment in all apolipoproteins and lipoproteins, with the exception of HDL cholesterol, which remained stable, whereas in men older

Table 1—Anthropometric, lifestyle, and metabolic characteristics of study subjects

	Women	Men	P
n	556	461	
Age (years)	45 ± 8	44 ± 9	0.046
Waist (cm)	81 ± 11	93 ± 10	<0.0001
BMI (kg/m ²)	24.7 ± 4.1	26.1 ± 3.4	<0.0001
Body fat mass (%)	22.7 ± 9.3	19.3 ± 7.9	<0.0001
Systolic blood pressure (mmHg)	114 ± 13	122 ± 10	<0.0001
Diastolic blood pressure (mmHg)	73 ± 8	76 ± 7	<0.0001
IPAQ (% score 1/2/3)	19/44/37	20/42/38	NS
Alcohol intake (g/week)	54 ± 65	104 ± 97	<0.0001
Smokers (never/current/ex, %)	46/24/30	46/26/28	NS
Menopause (%)	25.9	—	—
Fasting plasma glucose (mmol/L)	4.96 ± 0.51	5.20 ± 0.49	<0.0001
M/I (μmol · min ⁻¹ · kg _{FFM} ⁻¹ · nM ⁻¹)	156 ± 66	126 ± 65	<0.0001
Adiponectin (mg/L)	9.97 ± 3.82	6.59 ± 2.59	<0.0001
Total cholesterol (mmol/L)	4.83 ± 0.87	4.93 ± 0.86	NS
LDL cholesterol (mmol/L)	2.83 ± 0.81	3.10 ± 0.77	<0.0001
HDL cholesterol (mmol/L)	1.57 ± 0.38	1.26 ± 0.30	<0.0001
Triglycerides (mmol/L)	0.94 ± 0.44	1.25 ± 0.70	<0.0001
FFA (mmol/L)	0.59 ± 0.21	0.47 ± 0.19	<0.0001
ApoA1 (mg/dL)	139 ± 28	126 ± 24	<0.0001
ApoA2 (mg/dL)	33 ± 11	34 ± 10	NS
ApoB (mg/dL)	123 ± 50	137 ± 57	<0.0001
ApoC3 (mg/dL)	17.4 ± 6.8	18.8 ± 8.9	<0.007
ApoE (mg/dL)	5.14 ± 1.90	5.30 ± 2.04	NS
ALT (units/L)	20 ± 14	21 ± 13	NS

Data entries are means ± SD. P refers to the statistical significance of Student *t* test or χ^2 . IPAQ, International Physical Activity Questionnaire.

than 50 years, a smaller (5%) increment in total and LDL cholesterol was observed.

In univariate analysis (Supplementary Table 1), insulin sensitivity was positively associated with HDL cholesterol and negatively with triglycerides. The strength of the association was higher for HDL cholesterol than triglycerides and tended to be higher in men than women (STDB = +23.3 ± 3.0 vs. +19.0 ± 2.7 and -21.6 ± 3.0 vs. -14.5 ± 2.9, respectively, $P < 0.0001$ for all). LDL cholesterol was inversely associated with M/I in both men and women with lower STDB values (-8.3 ± 3.1 and -6.9 ± 2.7, respectively, $P < 0.02$ for both) that became nonstatistically significant when adjusted for HDL cholesterol and triglycerides. In both men and women, ApoA1 (STDB = +8.6 ± 3.0 and +7.0 ± 2.8, respectively) showed a direct association with insulin sensitivity that, however, was not independent of HDL cholesterol (adjusted STDB = -2.4 ± 3.3 and +0.4 ± 2.9, $P = NS$ for both). ApoB and ApoE were strongly correlated with insulin sensitivity only in men (STDB = -31.9 ± 7.3 and -12.8 ± 2.9, $P < 0.0001$); however, neither resulted independently of plasma triglycerides and HDL cholesterol.

ApoC3, interestingly, that was negatively associated with M/I only in men (STDB = -8.6 ± 3.1), when adjusted for triglycerides became positively correlated with insulin sensitivity both in men and women (STDB = +6.9 ± 3.4 and +10.9 ± 3.1, $P < 0.05$ for both). ApoA2 was not correlated to M/I in either sex. Pre- and postmenopausal women had a superimposable pattern of associations.

The strength of the associations between insulin sensitivity and HDL cholesterol and triglycerides in multivariate analysis after progressive adjusting for potential confounders is shown in Fig. 1, where the predicted changes in M/I (± SEM) for one SD change of either plasma lipid are illustrated. After adjusting for age, center, and sex (model a), the predicted impact of HDL cholesterol and triglycerides was similar (STDB = +19.9 ± 1.9 and -20.4 ± 2.0, respectively); further adjustment for the cognate lipid (model b) and BMI (model c) approximately halved the estimate, and adjustment for physical activity and smoking had no additional effect (model d). We then introduced into the model variables that could be relevant as mechanisms, i.e., adiponectin for HDL and FFA for triglycerides. Whereas the

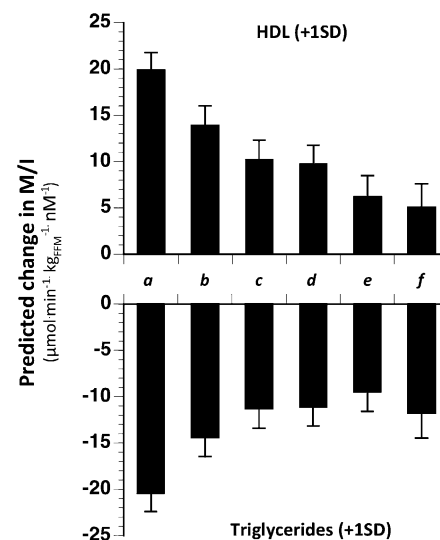


Figure 1—Estimated effects (and SE) of +1 SD of serum HDL cholesterol (top) and +1 SD of serum triglycerides (bottom) on insulin sensitivity (expressed as M/I). The lowercase letters indicate the different set of variables that were included in the different models: center, age, and sex (a); a + serum triglycerides for HDL or serum HDL cholesterol for triglycerides (b); b + BMI (c); c + smoking and physical activity (d); d + adiponectin for HDL and FFA for triglycerides (e); e + ApoA1 for HDL or ApoC3 for triglycerides (f).

former caused a 35% decline in the estimated effect of HDL on M/I, FFA entry into the model was associated with only a 15% reduction in the estimated effect of triglycerides (model e). We then evaluated the impact of the major associated lipoproteins; whereas ApoA1 caused a further minor reduction of the estimated effect of HDL, which became of borderline statistical significance ($P = 0.04$), adjusting for ApoC3 caused a 25% increase in the estimated impact of a change in triglycerides on M/I (model f).

In two stepwise multiple regression models using as independent variables the two sets (for HDL and triglycerides) included in Fig. 1, HDL cholesterol ranked after BMI, adiponectin, physical activity, triglycerides, and sex and before smoking, together explaining 35% of M/I variability; ApoA1 did not enter into the model; triglycerides ranked after BMI, HDL, sex, FFA, and physical activity and before smoking and ApoC3 (which had a positive coefficient), together explaining 35% of M/I variability.

The role of adiponectin in explaining the relationship between HDL cholesterol and insulin sensitivity also emerges from the strong association between the two

variables in both women and men ($r = 0.36$ and $r = 0.40$, respectively) (Fig. 2 and Supplementary Fig. 1). Of note, in a stepwise model predicting plasma adiponectin concentration in the entire cohort, HDL cholesterol ranked first (with $r^2 = 0.25$) followed by sex ($r^2 = 0.32$), waist girth ($r^2 = 0.34$), and age ($r^2 = 0.35$).

In an attempt to better understand the interaction of ApoC3 and triglyceride levels on M/I, we compared the regression slopes for the simple linear bivariate regression with the regression slopes when both variables were simultaneously included in a multivariate analysis. As shown in Fig. 2, after adjusting for triglycerides, ApoC3 was positively associated with M/I, whereas after adjusting for ApoC3, the association between M/I and serum triglycerides became steeper ($P < 0.001$ for slope comparison). In addition to the isolated effects of triglycerides

(negative) and ApoC3 (positive), the interaction was also statistically significant ($P < 0.02$) and negative. The positive association between ApoC3 and M/I once adjusted for triglycerides was present in both men and women (Fig. 2A, inset), whereas the change of the slope of triglycerides versus M/I after adjusting for ApoC3 (Fig. 2B, inset) showed a trend similar to the whole population but failed to reach statistical significance in either sex. Neither menopause in women nor age >50 years in men (entered in the model as covariates) modified by any extent the values of the slopes or the errors of the estimate.

To test whether by chronically limiting lipoprotein lipase activity ApoC3 might also reduce topic and/or ectopic fat deposition, we grouped subjects into tertiles of triglyceride with progressively higher ApoC3 enrichment (ApoC3 to triglycerides ratio) and then compared

body fat and serum alanine aminotransferase (ALT) concentration (as a proxy for fatty liver). As shown in Fig. 3, a higher ApoC3 enrichment of triglycerides was associated with lower body fat percent and lower serum ALT both in men and women.

CONCLUSIONS—The main findings of the current study are as follows: 1) the association of HDL cholesterol with insulin sensitivity is partially mediated by BMI, largely attributable to the tight correlation with plasma adiponectin, and unrelated to ApoA1 and 2) the association between triglycerides and insulin sensitivity is mediated by BMI and FFA and is unrelated to either ApoE or ApoB, whereas it is influenced by ApoC3, which appears to protect tissues from the negative impact of excess plasma lipid availability.

The lack of association between ApoA1 and insulin sensitivity in our study is in contrast with experimental studies in *ob/ob* mice, in which treatment with an ApoA1 mimetic (L-4F) was able to reduce fat depots, inflammation, and insulin resistance (2). The *ob/ob* mouse model is characterized by a high level of inflammation sustained by lipid peroxidation, which, in turn, can be effectively prevented by L-4F supplementation (2).

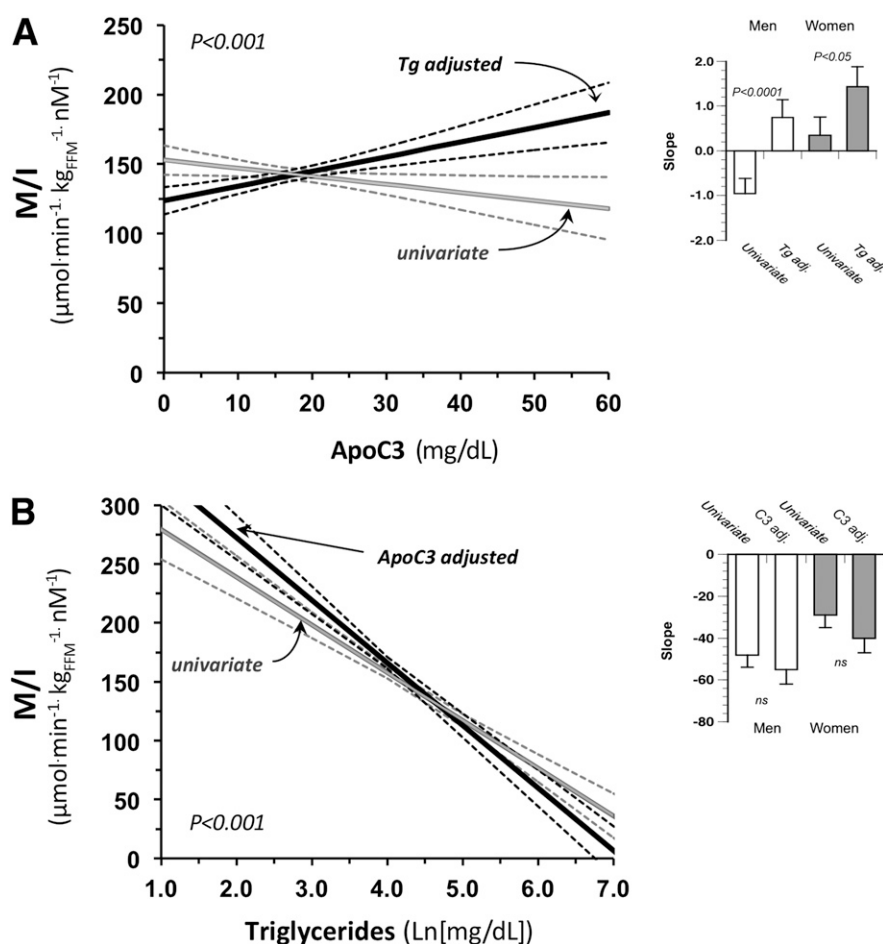


Figure 2—A: Regression lines and 95% CI for the slopes of insulin sensitivity (M/I) vs. ApoC3 (top) in univariate (gray) and after adjusting (black) for triglycerides. B: Regression lines and 95% CI for the slopes of insulin sensitivity (M/I) vs. triglycerides in univariate analysis (gray) and after adjusting for ApoC3 (black). In the inset, the values of the slopes are presented separately for men and women in both univariate and bivariate regression. P values indicate the level of statistical significance of the comparison between the slopes. Tg, triglyceride.

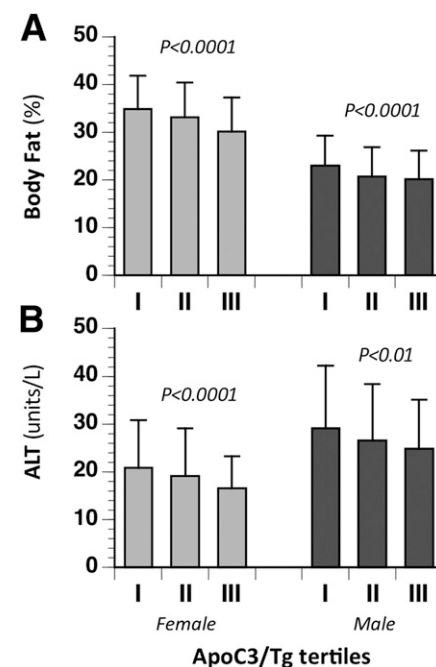


Figure 3—Body fat % (A) and serum ALT (B) in female (light gray histogram) and male (dark gray histogram) according to tertiles of the ratio ApoC3/triglycerides. P values indicate the statistical significance of the ANOVA. Tg, triglyceride.

In our population, the presence of low-grade inflammation, as estimated from high-sensitivity C-reactive protein >3 units/L, was present in only 7% of the participants and was unrelated to obesity, lipids, or insulin sensitivity (A. Natali, personal communication). In addition, the treatment of *ob/ob* mice with the ApoA1 mimetic was associated with an increase in plasma adiponectin, an effect that has been attributed to the decreased oxidative stress (25). We cannot exclude that in some specific, more extreme conditions (such as morbid obesity), pharmacological manipulations of ApoA1 might result in measurable effects on body fat and/or insulin sensitivity. Our observation that ApoA1 variability, at least within the physiologic range, in humans is unlikely to affect insulin sensitivity is consistent with a recent elegant experiment in type 2 diabetic patients, in whom a 4-h infusion of reconstituted HDL particles doubled serum ApoA1 concentrations and increased HDL cholesterol by 20% but had no effect on insulin sensitivity (26).

According to our analysis, the association between HDL cholesterol and insulin sensitivity is largely attributable to the fact that low HDL cholesterol is consistently accompanied by high triglycerides, obesity, low physical activity, and low plasma adiponectin. In this regard, it is interesting to note that the strength of the association between adiponectin and HDL cholesterol was much stronger than the ones observed with traditional HDL correlates such as triglycerides, sex, central fat distribution, alcohol, physical activity, age, and smoking. The fact that adiponectin has been shown in vivo in humans to reduce ApoA1 fractional catabolic clearance (27), and that HDL is able to induce adiponectin expression in visceral adipocytes in mice (4), is likely to justify the strong association between this lipoprotein and the adipokine.

Although experiments in genetically modified animals have shown that ApoE modulates diet-induced fat accumulation and insulin resistance (15), our data indicate that in humans, and within its physiological range of variability, ApoE does not seem to play a major role in either fat deposition or insulin action. In univariate analysis, ApoE was positively associated with indices of body fat (waist girth: $r = 0.19$, BMI: $r = 0.19$, % fat mass: $r = 0.16$) and negatively with M/I ($r = -0.13$), but also, very strongly, with serum triglycerides ($r = 0.41$). When serum triglycerides were included in multivariate models,

ApoE systematically lost statistical significance whereas triglycerides remained significantly associated with both obesity and insulin sensitivity. All the univariate associations of ApoE were stronger in men than in women, but the overall pattern of relationships in multivariate analysis was not different in the two sexes, nor did the use of the ApoC3/ApoE ratio add any additional power to our analysis.

With regard to ApoC3, our findings are consistent with the observations in genetically manipulated animals where this apolipoprotein appears to modulate insulin sensitivity by reducing the negative impact of triglycerides on insulin action and by limiting extravascular fat deposition (9). The epidemiological studies that have observed positive associations between ApoC3 and components of the metabolic syndrome (10,12) have failed to take into account the fact that the role of ApoC3 cannot be inferred without simultaneously adjusting for serum triglycerides. What appears to be of physiological importance is the lipoprotein relative enrichment in ApoC3, since the antilipolytic effect of this apolipoprotein is mainly physical, i.e., acting as a shield in the interaction between VLDL and endothelial lipoprotein lipase. By limiting triglyceride hydrolysis, ApoC3 reduces FFA availability. In our population, fasting ApoC3 levels had no direct influence on circulating FFA concentrations; the major determinants were body fatness, sex, and triglycerides. However, ApoC3 tertiles were associated with progressively lower values of the ratio FFA to triglycerides both in women (98 ± 45 , 82 ± 45 , and 66 ± 32 $\mu\text{Eq}/\text{mg}$) and men (69 ± 39 , 52 ± 29 , and 41 ± 26 $\mu\text{Eq}/\text{mg}$, both $P < 0.0001$). The phenomenon is expected to be even more important in the postabsorptive state, where plasma FFAs are mainly derived from lypolysis of circulating lipoprotein. On a chronic basis, a restrained lipoprotein lipolysis would reduce lipid transfer from the vascular to the extravascular space; this may translate into reduced tissue exposure to lipotoxicity. It is therefore not surprising that ApoC3 was an independent predictor of insulin sensitivity both alone (possibly by limiting fat depots) and in a negative interaction with serum triglycerides (protecting peripheral tissues from VLDL-derived FFAs). Despite these favorable metabolic interactions, ApoC3 might well be a cardiovascular risk factor (11) because it exerts a direct negative action on the vascular endothelium (28) and

because it lowers VLDL and IDL clearance, thereby prolonging their exposure to the vascular wall (6).

As our study is based on cross-sectional data, our conclusions on the nature of the observed associations are largely speculative. In order to limit the inherent risk in this approach, we carried out our analyses according to predefined hypotheses that were based on known physiology and experimental data. Therefore, we only make plausible inferences on the mechanisms underlying the regulation of tissue insulin sensitivity; clearly, ad hoc-designed clinical studies are needed to confirm our findings. In addition, the characteristics of our population (essentially healthy subjects) confine the validity of our conclusion to the physiological domain; it is possible that in some pathological conditions (e.g., severe hypertriglyceridemia, hyperglycemia, or morbid obesity), lipid regulation of insulin sensitivity might be different.

In conclusion, we cannot confirm that HDL lipoproteins directly exert a relevant role in modulating insulin sensitivity, the association between the two being explained mostly by confounders and in part by adiponectin. Conversely, we confirm that triglycerides exert a direct negative influence on insulin sensitivity and we provide strong support to the possibility that ApoC3, by controlling the lipid transfer from plasma to tissues, protects from the negative action of these substrates.

APPENDIX

RISC recruiting centers and investigators

Amsterdam, the Netherlands: R.J. Heine, J. Dekker, S. de Rooij, G. Nijpels, and W. Boersma. Athens, Greece: A. Mitrakou, S. Tournis, K. Kyriakopoulou, and P. Thomakos. Belgrade, Serbia: N. Lalic, K. Lalic, A. Jotic, L. Lukic, and M. Civcic. Dublin, Ireland: J. Nolan, T.P. Yeow, M. Murphy, C. DeLong, G. Neary, M.P. Colgan, and M. Hatunic. Frankfurt, Germany: T. Konrad, H. Böhles, S. Fuellert, F. Baer, and H. Zuchhold. Geneva, Switzerland: A. Golay, E. Harsch Bobbioni, V. Barthassat, V. Makoundou, T.N.O. Lehmann, and T. Merminod. Glasgow, U.K.: J.R. Petrie (now Dundee), C. Perry, F. Neary, C. MacDougall, K. Shields, and L. Malcolm. Kuopio, Finland: M. Laakso, U. Salmenniemi, A. Aura, R. Raisanen, U. Ruotsalainen, T. Sistonen, M. Laitinen, and H. Saloranta. London, U.K.: S.W. Coppack, N. McIntosh, J. Ross, L. Pettersson, and

P. Khadobaksh. Lyon, France: M. Laville, F. Bonnet (now Rennes), A. Brac de la Perriere, C. Louche-Pelissier, C. Maitrepierre, J. Peyrat, S. Beltran, and A. Serusclat. Madrid, Spain: R. Gabriel, E.M. Sánchez, R. Carraro, A Friera, and B. Novella. Malmö, Sweden (1): P. Nilsson, M. Persson, and G. Östling. Malmö, Sweden (2): O. Melander and P. Burri. Milan, Italy: P.M. Piatti, L.D. Monti, E. Setola, E. Galluccio, F. Minicucci, and A. Colleluori. Newcastle upon Tyne, U.K.: M. Walker, I.M. Ibrahim, M. Jayapaul, D. Carman, C. Ryan, K. Short, Y. McGrady, and D. Richardson. Odense, Denmark: H. Beck-Nielsen, P. Staehr, K. Hojlund, V. Vestergaard, C. Olsen, and L. Hansen. Perugia, Italy: G.B. Bolli, F. Porcellati, C. Fanelli, P. Lucidi, F. Calcinaro, and A. Saturni. Pisa, Italy: E. Ferrannini, A. Natali, E. Muscelli, S. Pinnola, M. Kozakova, A. Casolaro, and B.D. Astiarraga. Rome, Italy: G. Mingrone, C. Guidone, A. Favuzzi, and P. Di Rocco. Vienna, Austria: C. Anderwald, M. Bischof, M. Promintzer, M. Krebs, M. Mandl, A. Hofer, A. Luger, W. Waldhäusl, and M. Roden.

Core laboratories and reading centers

Lipids (Dublin, Ireland): P. Gaffney, J. Nolan, and G. Boran. Apolipoprotein (Pisa, Italy): S. Baldi. Hormones (Odense, Denmark): C. Olsen, L. Hansen, and H. Beck-Nielsen. Actigraph (Villejuif, France): B. Balkau and L. Mhamdi. Data management (Villejuif, France and Padova and Pisa, Italy): B. Balkau, A. Mari, L. Mhamdi, L. Landucci, S. Hills, and L. Mota. Mathematical modeling and website management (Padova, Italy): A. Mari, G. Pacini, C. Cavaggion, and A. Tura. Coordinating office (Pisa, Italy): S.A. Hills, L. Landucci, and L. Mota.

Project management board

B. Balkau (Villejuif, France), S.W. Coppack (London, U.K.), J.M. Dekker (Amsterdam, the Netherlands), E. Ferrannini (Pisa, Italy), A. Mari (Padova, Italy), A. Natali (Pisa, Italy), and M. Walker (Newcastle, England).

Further information on the RISC Study and participating centers can be found at www.egir.org.

Acknowledgments—The RISC Study was supported by European Union grant QLGI-CT-2001-01252.

Additional support was provided by AstraZeneca (Sweden). The European Group for the Study of Insulin Resistance group activities

were supported by an unrestricted research grant from Merck Serono (France). No other potential conflicts of interest relevant to this article were reported.

S.B. supervised the apolipoprotein laboratory, designed the “RISC apolipoprotein” study subproject, collected and analyzed data, and wrote the results and discussion. F.B., M.L., C.M., L.M., and K.H. recruited subjects, performed clinical investigations, collected data, and revised the manuscript. E.F. designed the RISC Study and revised the manuscript. A.N. analyzed data and wrote the manuscript. S.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am* 2006;35:491–510, vii–viii
2. Peterson SJ, Drummond G, Kim DH, et al. L-4F treatment reduces adiposity, increases adiponectin levels, and improves insulin sensitivity in obese mice. *J Lipid Res* 2008;49:1658–1669
3. Ruan X, Li Z, Zhang Y, et al. Apolipoprotein A-I possesses an anti-obesity effect associated with increase of energy expenditure and up-regulation of UCP1 in brown fat. *J Cell Mol Med* 2011;15:763–772
4. Van Linthout S, Foryst-Ludwig A, Spillmann F, et al. Impact of HDL on adipose tissue metabolism and adiponectin expression. *Atherosclerosis* 2010; 210:438–444
5. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423:762–769
6. Gangabadage CS, Zdunek J, Tessari M, Nilsson S, Olivecrona G, Wijmenga SS. Structure and dynamics of human apolipoprotein CIII. *J Biol Chem* 2008;283: 17416–17427
7. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol* 1999;19:472–484
8. Ebara T, Ramakrishnan R, Steiner G, Shachter NS. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. *J Clin Invest* 1997;99:2672–2681
9. Duivenvoorden I, Teusink B, Rensen PC, Romijn JA, Havekes LM, Voshol PJ. Apolipoprotein C3 deficiency results in diet-induced obesity and aggravated insulin resistance in mice. *Diabetes* 2005;54: 664–671
10. Olivieri O, Bassi A, Stranieri C, et al. Apolipoprotein C-III, metabolic syndrome,

and risk of coronary artery disease. *J Lipid Res* 2003;44:2374–2381

11. Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation* 2000;102:1886–1892
12. Florez H, Mendez A, Casanova-Romero P, et al. Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. *Atherosclerosis* 2006;188:134–141
13. Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in non-alcoholic fatty liver disease. *N Engl J Med* 2010;362:1082–1089
14. van der Ham RL, Alizadeh Dehnavi R, Berbée JF, et al. Plasma apolipoprotein CI and CIII levels are associated with increased plasma triglyceride levels and decreased fat mass in men with the metabolic syndrome. *Diabetes Care* 2009;32:184–186
15. Gao J, Katagiri H, Ishigaki Y, et al. Involvement of apolipoprotein E in excess fat accumulation and insulin resistance. *Diabetes* 2007;56:24–33
16. Söderlund S, Watanabe H, Ehnholm C, Jauhiainen M, Taskinen MR. Increased apolipoprotein E level and reduced high-density lipoprotein mean particle size associate with low high-density lipoprotein cholesterol and features of metabolic syndrome. *Metabolism* 2010;59:1502–1509
17. Bach-Ngohou K, Ouguerram K, Nazih H, et al. Apolipoprotein E kinetics: influence of insulin resistance and type 2 diabetes. *Int J Obes Relat Metab Disord* 2002;26: 1451–1458
18. Meigs JB, Ordovas JM, Cupples LA, et al. Apolipoprotein E isoform polymorphisms are not associated with insulin resistance: the Framingham Offspring Study. *Diabetes Care* 2000;23:669–674
19. Després JP, Verdon MF, Moorjani S, et al. Apolipoprotein E polymorphism modifies relation of hyperinsulinemia to hypertriglyceridemia. *Diabetes* 1993;42:1474–1481
20. Valdez R, Howard BV, Stern MP, Haffner SM. Apolipoprotein E polymorphism and insulin levels in a biethnic population. *Diabetes Care* 1995;18:992–1000
21. Elosua R, Demissie S, Cupples LA, et al. Obesity modulates the association among APOE genotype, insulin, and glucose in men. *Obes Res* 2003;11:1502–1508
22. Hills SA, Balkau B, Coppack SW, et al.; EGIR-RISC Study Group. The EGIR-RISC Study (The European Group for the Study of Insulin Resistance: Relationship between Insulin Sensitivity and Cardiovascular Disease Risk): I. Methodology and objectives. *Diabetologia* 2004;47:566–570
23. Frystyk J, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A. Increased serum adiponectin levels in type 1 diabetic patients

- with microvascular complications. *Diabetologia* 2005;48:1911–1918
24. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214–E223
 25. L'Abbate A, Neglia D, Vecoli C, et al. Beneficial effect of heme oxygenase-1 expression on myocardial ischemia-reperfusion involves an increase in adiponectin in mildly diabetic rats. *Am J Physiol Heart Circ Physiol* 2007;293:H3532–H3541
 26. Drew BG, Duffy SJ, Formosa MF, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. *Circulation* 2009;119:2103–2111
 27. Vergès B, Petit JM, Duvillard L, et al. Adiponectin is an important determinant of apoA-I catabolism. *Arterioscler Thromb Vasc Biol* 2006;26:1364–1369
 28. Kawakami A, Yoshida M. Apolipoprotein CIII links dyslipidemia with atherosclerosis. *J Atheroscler Thromb* 2009;16:6–11