Serum IF1 concentration is independently associated to HDL levels and to coronary heart disease: the GENES study

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Abstract HDL is strongly inversely related to cardiovascular risk. Hepatic HDL uptake is controlled by ecto-F$_1$-ATPase activity, and potentially inhibited by mitochondrial inhibitor factor 1 (IF1). We recently found that IF1 is present in serum and correlates with HDL-cholesterol (HDL-C). Here, we have evaluated the relationship between circulating IF1 and plasma lipoproteins, and we determined whether IF1 concentration is associated with the risk of coronary heart disease (CHD). Serum IF1 was measured in 648 coronary patients ages 45–74 and in 669 matched male controls, in the context of a cross-sectional study on CHD. Cardiovascular risk factors were documented for each participant, including lifestyle habits and biological and clinical markers. In controls, multivariate analysis demonstrated that IF1 was independently positively associated with HDL-C and apoA-I ($r = 0.27$ and 0.28, respectively, $P < 0.001$) and negatively with triglycerides ($r = -0.23$, $P < 0.001$). Mean IF1 concentration was lower in CHD patients than in controls ($0.43$ mg/l and $0.53$ mg/l, respectively, $P < 0.001$). In multivariate analyses, following adjustments on cardiovascular risk factors or markers, IF1 was negatively related to CHD ($P < 0.001$). This relationship was maintained even after adjustment for HDL-C or apoA-I. This study identifies IF1 as a new determinant of HDL-C that is inversely associated with CHD. Serum IF1 concentration is independently associated to HDL levels and to coronary heart disease: the GENES study. J. Lipid Res. 2013, 54: 2550–2558.

Supplementary key words
HDL-cholesterol • coronary heart disease • ATP-synthase inhibitor factor 1 • nucleotide receptor P2Y$_{13}$ • inhibitor factor 1

Despite considerable advances in the treatment of cardiovascular diseases, they remain among the leading causes of death in developed countries. Several large-scale prospective studies evidenced an independent inverse association of HDL-cholesterol (HDL-C) levels with risk of coronary heart disease (CHD) (1). Accordingly, studies in genetically modified animals, and in patients with rare disorders of HDL metabolism, support a causal relationship between low HDL and development of atherosclerotic vascular disease (1, 2). A low level of HDL-C (generally considered as <40 mg/dl in men and <50 mg/dl in women) remains predictive of future cardiovascular risk, even when the concentration of LDL-cholesterol (LDL-C) has reached low levels upon treatment with statins (3). Research to understand the protective effects of HDL against coronary artery disease (CAD) has yielded a large number of likely beneficial actions of HDL particles and their components, such as antiinflammatory, antithrombotic, antioxidative, and cytoprotective effects (1). However, the best-characterized protective action of HDL is their central contribution in a process called reverse cholesterol transport (RCT), a process whereby cholesterol is transported from peripheral cells to the liver for further excretion into bile and feces. Thus, these pleiotropic cardioprotective functions of HDL, supported by epidemiological studies, led to the idea that therapies aiming at enhancing plasma HDL levels would...
be antiatherogenic and protective against cardiovascular events. However, this issue was revised with recent results from large clinical trials showing that some mechanisms that enhance HDL levels are not necessarily beneficial toward atherosclerosis (4, 5). HDL is highly heterogeneous with subclasses that differ in composition and physical properties, and display different antiatherogenic properties (6). Moreover, several environmental factors, such as tobacco and alcohol consumption, physical activity, or inflammation, are known to influence plasma HDL levels and functions (1). Given the complexity of the HDL system, it has emerged that a single measurement of HDL-C level often fails to provide a reliable prediction of HDL biological activities (7). Therefore, new biological markers reflecting metabolic or vascular activities of HDL lipoproteins are needed to better evaluate patients’ cardiovascular risk or to evaluate their responsiveness to emergent HDL-related therapies (2).

In this context, we previously demonstrated the presence of a protein complex, related to mitochondrial ATP-synthase, on the surface of human hepatocytes, behaving as a high-affinity receptor for HDL apolipoprotein A-I (apoA-I). ApoA-I binds to cell surface ATP-synthase (namely, ecto-F$_1$-ATPase), stimulating extracellular ATP hydrolysis into ADP (8). ADP further activates the nucleotide receptor P2Y$_{13}$, resulting in HDL endocytosis (9). We and others (10, 11) described that P2Y$_{13}$-knockout mice displayed altered HDL hepatic catabolism and impaired RCT, but HDL levels were maintained or slightly decreased, supporting the emerging concept that steady-state serum HDL levels do not necessarily reflect the ability of HDL to promote RCT (12).

Inhibitory factor 1 (IF1) is a mitochondrial protein of 81 amino acids (NCBI Reference Sequence: NM_016311.4; NP_057395.1) that specifically inhibits the ATPase activity of the mitochondrial F$_{0}$F$_{1}$-ATP synthase (13). Using human hepatocytes and perfused rat liver, we previously demonstrated that exogenous recombinant IF1 also inhibits F$_{1}$-ATPase / P2Y$_{13}$-mediated HDL uptake (8). In addition, we recently described the presence of constitutive IF1 in human serum, and we developed a specific immunosassay for this compound (14). First measurements in 100 normolipidemic subjects have shown a positive association of IF1 level with HDL-C (14). In the present work, IF1 measurements were extensively performed in the framework of a case-control study on CHD. The results argue in favor of IF1 being a new determinant of HDL levels and a potential biomarker of cardiovascular risk. IF1 measurements might be particularly relevant for CHD risk assessment in individuals with low HDL levels.

METHODS

Study sample

The Génétique et Environnement en Europe du Sud (GENES) study is a case-control study designed to assess the role of genetic, biological, and environmental determinants in the occurrence of CHD. All participants signed an informed consent form. The study protocol was approved by the local ethics committee (CCPRPB, Toulouse / Sud-Ouest, file #1-99-48, Feb 2000). A biological sample collection has been constituted (declared as DC-2008-463 #1 to the Ministry of Research and to the regional Health authority). As previously described, cases were stable CHD patients, ages 45–74, living in the Toulouse area (southwest France) and prospectively recruited from 2001 to 2004 after admission to the Cardiology Department, Toulouse University Hospital, for cardiovascular examination and referred for evaluation and management of their CHD (15, 16). Stable CHD was defined by a previous history of acute coronary syndrome, a previous history of coronary artery revascularization, a documented myocardial ischemia, a stable angina, or the presence at coronary angiography of a coronary stenosis of 50% or more. Patients who had presented an acute coronary episode during the past eight days were not included in the study, because they were considered unstable. During the same period, male controls, ages 45–74, were selected from the general population using electoral rolls. Stratification into decadal age groups was used to approximately match the age distribution of the people with and without CHD.

Data collection

Male participants with and without CHD were examined in the morning after an overnight fast. Patients with stable CHD (cases) were examined in the clinics of the Cardiology Department, Toulouse University Hospital and underwent a coronary angiography. Control subjects were examined in the same health center. The medical examination started with standardized face-to-face interviews performed by a trained physician using standardized methods. During this interview, participants received oral and written information on the aim of the study and signed a consent form. Then a blood sample was taken from each participant. Each participant then completed standardized questionnaires, lasting for about 40 min, covering age, socioeconomic variables, educational level (number of years spent at school), smoking status, alcohol consumption, physical activity, information on cardiovascular risk factors, and past medical (personal and parental) history. The medical examination was continued with anthropometric and clinical measurements. Smoking status was classified as current smokers, past smokers who had quit smoking for at least three years before the interview, and nonsmokers. For current smokers, cigarette consumption was also recorded as the average number of cigarettes per day. Alcohol consumption was assessed using typical weekly patterns. Physical activity (PA) was categorized into four levels: no PA, light PA for 20 min once a week, moderate PA for 20 min twice a week, and intense PA for 20 min at least three times a week or more. In this study, PA was considered and studied as a dichotomous variable: PA > (“high”) or ≤ (“low”) 20 min, once a week. Presence of dyslipidemia, diabetes or hypertension was assessed from the subjects’ current treatments. In those with CHD, their medication at discharge was also considered. Anthropometrical measurements included waist circumference and body mass index (BMI). Blood pressure and heart rate were measured with an automatic sphygmomanometer. Right arm blood pressure was measured twice, and the average value was recorded. Measurements were performed after a 5 min rest. Blood samples were analyzed for serum total cholesterol, HDL-C, triglycerides (TG), glucose, γ-glutamyltranspeptidase (γ-GT), and sensitive C-reactive protein (CRP) with enzymatic reagents on an automated analyzer (Hitachi 912, Roche Diagnostics, Meylan, France). ApoAI, apoB, and lipoprotein (a) ([Lp(a)]) were assayed with an immunoturbidimetric method on an automated analyzer (Roche Diagnostics, France). LDL-C was calculated using the Friedewald formula, with VLDL-cholesterol (VLDL-C) (g/l) = TG (g/l)/5, as long as TG concentration was below 4 g/l (17). Lipoproteins containing apoB and either apoCIII (Lp B:CIII) or apoE (Lp B:E) were assayed by a specific immunoelctrodiffusion assay (Sebia, France).
Immunossay of IF1

A competitive immunossay was developed to quantify IF1 in human sera as previously described (14). Repeatability within the same day and reproducibility (10 measurements over a six-month period) gave variation coefficients of 6–7%. An average recovery of 96.8% was measured in dilution experiments. Resistance to freezing and thawing was assessed on different occasions with a 95–103% agreement between measurements. With regard to possible cross-reactivity in this immunoassay, 95–103% agreement between measurements. With regard to possible cross-reactivity in this immunoassay, resistance to freezing and thawing was assessed on different occasions with a 95–103% agreement between measurements.

Statistical analyses

In the control group, associations of IF1, HDL-C, and apoA-I with clinical characteristics and biological markers were tested using Spearman’s rank correlations. Independent associations of IF1 with HDL-C and apoA-I were analyzed using a multivariate linear regression model. Two separate regression analyses of HDL-C on IF1 and apoA-I were performed. Conversely, regression analyses of IF1 on HDL-C or apoA-I and other lipoprotein variables were also made. A stepwise selection method was used to avoid confounding variables. The F statistics for a variable to be added in the model had to be significant at P<0.10. Variables were further maintained in the model when the F statistic was significant at P<0.05. However, although associations with LDL-C were not significant, LDL-C, as an established lipid risk factor, was forcibly included in final models. For each quantitative variable, regression analyses were performed with polynomial models to look for possible nonlinear relationships.

In the tables, data are presented as percentages for qualitative variables and as means with standard deviations for quantitative ones. The chi-square test was used to compare the distribution of qualitative variables between cases and controls. The mean values of quantitative variables were compared by Student t-test. Shapiro-Wilk’s test was used to test the normality of distribution of residuals and Levene test to test the homogeneity of variances. When the basic assumptions of Student t-test were not satisfied, the data were logarithmically transformed or subjected to a Wilcoxon-Mann-Whitney test.

Two separate multivariate logistic regressions were carried out to analyze the statistical association of IF1, HDL-C, and apoA-I quartiles with CHD. Adjustments were made systematically for classical cardiovascular risk factors: (treatment for) dyslipidemia, hypertension, diabetes, smoking, and also for physical activity, alcohol consumption, C reactive protein, Lp(a), and triglycerides, which were found associated with CHD (supplemental Table IV). For the IF1 logistic regression model, a further adjustment was made for HDL-C or apoA-I. Distributions of IF1, HDL-C, and apoA-I were dichotomized (the median cut-off value was 0.47 mg/l for IF1, 0.43 g/l for HDL-C, and 1.37 g/l for apoA-I) to test the statistical interaction between IF1 and HDL-C or apoA-I. The Hosmer-Lemeshow chi-square statistic was used to test the statistical interaction between IF1 and HDL-C or apoA-I.

The performance of the final model was evaluated by analysis of the area under the receiver operating characteristic (ROC) curve (AUC). The internal validity of the prediction model was evaluated by bootstrapping (18) using 100 random samples. ROC curves were constructed, and models with and without IF1 or HDL-C, separately or in combination, were compared (19). Analyses were two-tailed, and P<0.05 was considered to be significant.

RESULTS

Serum IF1 levels in the general population

Plotting of IF1 levels in the general male population (669 controls ages 45–74) demonstrated a normal distribution with a median value at 0.53 ± 0.15 mg/l and a 95% confidence interval (CI) of 0.24–0.82 mg/l (Fig. 1 and Table 1). Serum IF1 was positively correlated with HDL-C and apoA-I, and negatively with triglycerides and triglyceride-containing lipoproteins (Fig. 2 and Table 2). Both HDL-C and apoA-I regularly increased as a function of quintiles of IF1 in the control population (supplemental Table 1). Although the GENES study dealt only with male subjects, it is most likely that the relationship between serum IF1 and lipids also exists among women. In a preliminary approach, we measured IF1 concentrations among 70 normolipemic women who participated in a population study in the same geographical area. Mean IF1 level was 0.52 mg/l ± 0.21. A positive correlation with HDL-C and a negative one with TG concentration were also observed (A. Genoux, unpublished observations).

Correlations between IF1 levels and individual metabolic parameters or cardiovascular risk markers were investigated and compared with equivalent correlations for HDL-C and apoA-I (Table 2). All three parameters (IF1, HDL-C, and apoA-I) were negatively correlated with BMI and waist circumference. Environmental factors, such as cigarette smoking, alcohol consumption, physical activity, and an inflammatory condition, as documented by elevated CRP, displayed expected correlations with HDL-C or apoA-I, but correlations with IF1 were absent, or much weaker in the case of alcohol (Table 2). This suggests that IF1 levels, for most part, are little influenced by environmental variables that are known to affect HDL-C or apoA-I levels. This hypothesis was supported by multivariate analyses conducted among control subjects. First, we developed...
Serum IF1 levels and coronary heart disease

All characteristics of CHD and control individuals were considered (Table 1). Increased prevalence of classical risk factors was recorded in CHD individuals: hypertension, diabetes, dyslipidemia, and smoking habits. BMI and waist circumference were higher in CHD-affected than in control individuals, while educational level and physical activity were lower. Averages of alcohol consumption were not significantly different between cases and controls. However, alcohol patterns were different, with a higher percentage of moderate alcohol consumption (<40 g/day) in controls and, conversely, a higher percentage of both abstinent and excessive drinkers (≥40 g/day) in cases (data not shown). Among metabolic markers, total or LDL-C were lower in CHD individuals, probably reflecting effects of lipid-lowering drugs in patients. However, CHD individuals displayed hyperglycemia, higher levels of triglyceride-containing lipoproteins and Lp(a), increased CRP, and lower HDL markers. Altogether, 50% of CAD individuals had features of metabolic syndrome, according to the NCEP-ATPIII definition versus 18% of control subjects. Average IF1 was 0.43 ± 0.13 in CHD individuals versus 0.53 ± 0.15 mg/l in controls (P < 0.001, Table 1). We also determined IF1 levels in cases and controls taking or not taking treatments for dyslipidemia, diabetes, or hypertension, and we observed no impact of those treatments on serum IF1 (supplemental Table III).

A prior logistic regression analysis of established risk factors associated with occurrence of CHD in our study identified smoking, diabetes, dyslipidemia or hypertension, elevated CRP, TG, Lp(a), and low physical activity (supplemental Table IV). A trend to positive association was observed for alcohol. Thus, all these factors were taken into account for further statistical adjustments. Logistic regression analyses were conducted regarding the CHD status and studying HDL-C, apoA-I, and IF1 as explanatory variables, following multiple adjustments on cardiovascular risk factors and markers having shown significant differences between the two subpopulations. All three parameters were divided into quartiles, and odds ratios (OR) with 95% CI were determined (Table 4). A negative association with CHD was recorded with increasing levels of the three

### Table 1. Clinical and biological characteristics of the study population

| Characteristics                  | Cases (n = 648) | Controls (n = 669) | P     |
|----------------------------------|----------------|-------------------|-------|
| Age (year)                       | 60.4 (±7.9)    | 59.2 (±8.3)       | 0.007 |
| School (years of education)      | 9.6 (±2.9)     | 13.1 (±4.1)       | 0.001 |
| Alcohol (g/day)                  | 28.1 (±30.6)   | 24.0 (±22.4)      | 0.37  |
| Waist (cm)                       | 99.0 (±10.9)   | 95.3 (±9.6)       | 0.001 |
| BMI (kg/m²)                      | 27.4 (±4.1)    | 26.8 (±5.3)       | 0.002 |
| SBP (mmHg)                       | 139.7 (±20.6)  | 136.9 (±16.0)     | 0.002 |
| Heart rate (beat/min)            | 65.8 (±12.0)   | 65.4 (±9.3)       | 0.41  |
| Glucose (mmol/l)                 | 5.94 (±2.1)    | 5.47 (±1.1)       | 0.001 |
| Triglycerides (g/l)              | 1.70 (±1.01)   | 1.23 (±0.76)      | 0.001 |
| Total cholesterol (g/l)          | 2.01 (±0.44)   | 2.25 (±0.37)      | 0.001 |
| LDL-C (g/l)                      | 1.29 (±0.38)   | 1.51 (±0.34)      | 0.001 |
| HDL-C (g/l)                      | 0.39 (±0.11)   | 0.50 (±0.11)      | 0.001 |
| γ-GT (IU/l)                      | 65.5 (±74.8)   | 45.9 (±52.8)      | 0.001 |
| Lp(a) ≥0.30 versus <0.30 g/l (%) | 52.6           | 34.1              | 0.001 |
| CRP ≥5 mg/l versus < (%)        | 53.6           | 16.6              | 0.001 |
| Metabolic syndrome (NCEP-ATPIII %)| 50.4           | 17.8              | 0.001 |
| Treatment for diabetes (%)       | 25.0           | 6.4               | 0.001 |
| Treatment for dyslipidemia (%)   | 65.6           | 23.6              | 0.001 |
| Treatment for hypertension (%)   | 43.8           | 20.6              | 0.001 |
| Cigarettes (cig/day)             | 3.2 (±8.1)     | 1.9 (±6.4)        | 0.001 |
| Smoking habits (%)               |                |                   | 0.001 |
| Current smoking                  | 20.4           | 12.7              |       |
| Past smoking                     | 61.9           | 53.2              |       |
| Never smoking                    | 17.7           | 34.1              |       |
| Physical activity (high level) (%)| 11.5           | 34.2              | 0.001 |
| IF1 (mg/l)                       | 0.43 (±0.13)   | 0.53 (±0.15)      | 0.001 |

Data are expressed as mean (± SD) or percentage.

*Analyses performed on log-transformed data.

*“High” physical activity during 20 min at least twice a week versus “low” physical activity once a week or less.

multiple regression models that included IF1 as an explanatory variable of HDL-C or apoA-I (Table 3). In model 1, which explained 34.2% of HDL-C variability, triglyceride concentration was the major determinant of HDL-C levels (56.3%), being inversely related, followed by serum IF1, positively associated (10.1%). In model 2, which explained 21.2% of apoA-I variability, alcohol consumption and serum IF1 were the strongest positive contributors of apoA-I variability (27.0% and 30.5%, respectively). As expected, BMI, tobacco smoking, or an inflammatory condition was a negative determinant, whereas physical activity was positively associated. Serum IF1 was then studied as the dependent variable in multiple regression analyses. Two models were developed that included either HDL-C or apoA-I as explanatory variables (supplemental Table II). The two models explained 10.4% and 12.3%, respectively, of IF1 variability. HDL-C or apoA-I were the strongest contributors of the models, followed by triglyceride and apoB levels as negative determinants. Alcohol consumption also contributed positively and significantly to IF1 variability, although to a lesser extent (supplemental Table II).
instead of HDL-C, only quartile 4 of IF1 appeared associated with a significant reduction (−60%) (Table 4). To ensure that our results on IF1 were not influenced by the proximity of a coronary event, analyses were resumed after excluding those patients having presented an acute event in the last three months, but OR were identical to those in the whole population (supplemental Table V). Quartile ranking was performed to provide a convenient illustration of those relationships, but significant associations were also found when HDL-C, apoA-I, and IF1 were considered as continuous variables (data not shown). The linearity of the relationships was verified. Thus, multivariate analyses argues in favor of IF1 being independently negatively associated with CHD. A statistical interaction existed on CHD risk, between IF1 and HDL-C or apoA-I levels (P = 0.04 and P = 0.009, respectively, Table 5). To illustrate this interaction, IF1, HDL-C, and apoA-I concentrations were considered from both sides of their medians, and the ORs were calculated. At low levels of HDL-C (<0.43 g/l), an elevated IF1 (≥0.47 mg/l) conferred a 69% reduction of the OR for CHD (P < 0.001). A similar decrease was observed in the reverse situation; i.e., at low levels of IF1 but with elevated HDL-C. However, risk reduction was significantly greater (−86%, P < 0.001) when both markers were above medians. Combination of elevated IF1 (≥0.47 mg/l) and HDL-C (≥0.43 g/l) was associated with an OR reduction of greater magnitude than either one of the two markers alone. At low levels of apoA-I (<1.37 g/l), an elevated IF1 (≥0.47 mg/l) conferred a 68% risk reduction (P < 0.001). Risk reduction was significantly greater (P < 0.001) when both markers were above medians (−90%). However, an elevated apoA-I by itself achieved a similar reduction of CHD occurrence. Pairwise comparisons (Table 5) clearly show that the beneficial effect of elevated IF1 (above median) in terms of reduced association to CHD is more pronounced at low levels of HDL-C or apoA-I (below median). Again, data were considered across medians for presentation clarity, but similar relationships were observed for continuous variables (not shown).

To further explore the associations with CHD, ROC curves were constructed using classical cardiovascular risk factors and markers with or without IF1, HDL-C, or IF1+HDL-C (supplemental Fig. I). Classical cardiovascular risk factors and markers having shown differences between the two sub-populations included (treatment for) dyslipidemia, hypertension, and diabetes; smoking, physical activity, and alcohol consumption; and CRP, Lp(a), and TG. The AUC for this “basal panel” was 0.866 as regards CHD. AUC was increased after addition to the model of either IF1 (AUC = 0.880, P < 0.001 for the difference) or HDL-C (AUC = 0.892, P < 0.001). Furthermore, in the latter case (basal panel + HDL-C), inclusion of IF1 concentration still significantly increased AUC, compared with HDL-C alone (0.897 with HDL-C+IF1 versus 0.892 with HDL-C, P < 0.01, supplemental Fig. I).

**DISCUSSION**

A few years ago, we found that membrane ecto-F$_1$-ATPase, an enzyme complex related to mitochondrial ATP-synthase,
was a high-affinity receptor for apoA-I, leading secondarily to liver uptake of HDL particles (8). More recently, we detected the presence in human serum of IF1, the natural inhibitor of mitochondrial F$_1$-ATPase, and we developed a specific immunoassay for serum IF1 (14). In the present study, for the first time, large-scale measurements of serum IF1 were performed in the context of a case-control study on CHD. In control subjects taken from the general population, we again found correlations between serum IF1 and other cardiovascular risk factors in controls.

| Characteristics | IF1 (mg/l) | HDL-C (g/l) | ApoA-I (g/l) |
|-----------------|------------|-------------|-------------|
| Age (years)     | −0.02 (−0.10; 0.05) | 0.03 (−0.04; 0.11) | 0.05 (−0.02; 0.13) |
| Alcohol (g/day) | 0.09 (0.02; 0.16)* | 0.20 (0.12; 0.27)*** | 0.28 (0.21; 0.31)*** |
| Smoking (cig/day)| −0.03 (−0.11; 0.05) | −0.10 (−0.17; −0.02)* | −0.10 (−0.17; −0.02)* |
| Physical activity score | 0.01 (−0.07; 0.08) | 0.20 (0.15; 0.27)*** | 0.09 (0.02; 0.17)* |
| BMI (kg/m$^2$)  | −0.17 (−0.24; −0.09)*** | −0.35 (−0.40; −0.26)*** | −0.29 (−0.27; −0.12)*** |
| Waist (cm)      | −0.15 (−0.22; −0.08)*** | −0.34 (−0.41; −0.27)*** | −0.17 (−0.24; −0.10)*** |
| SBP (mmHg)      | −0.02 (−0.09; 0.06) | −0.08 (−0.15; 0.01)* | 0.01 (−0.08; 0.08) |
| Heart rate (beat/min) | −0.04 (−0.12; 0.03) | −0.07 (−0.14; 0.01) | −0.05 (−0.12; 0.03) |
| Total cholesterol (g/l) | −0.07 (−0.14; 0.01) | 0.16 (0.09; 0.24)*** | 0.23 (0.16; 0.30)*** |
| LDL-C (g/l)     | −0.07 (−0.15; 0.01) | 0.01 (−0.07; 0.08) | 0.03 (−0.05; 0.10) |
| HDL-C (g/l)     | 0.24 (0.16−0.31)*** | 0.82 (0.80; 0.84)*** |
| ApoA-I (g/l)    | 0.27 (0.20−0.34)*** | 0.82 (0.80; 0.84)*** |
| ApoB (g/l)      | −0.17 (−0.24; −0.09)*** | −0.15 (−0.22; −0.08)*** | −0.07 (−0.14; 0.01) |
| Triglyceride (g/l) | −0.25 (−0.32; −0.17)*** | −0.48 (−0.54; −0.42)*** | −0.19 (−0.26; −0.12)*** |
| LpB:CIII (mg/l) | −0.14 (−0.32; −0.17)*** | −0.34 (−0.41; −0.27)*** | −0.06 (−0.14; 0.02) |
| LpB:E (mg/l)    | −0.26 (−0.33; −0.19)*** | −0.25 (−0.32; −0.18)*** | −0.21 (−0.28; −0.13)*** |
| Lp(a) (g/l)     | 0.06 (−0.02; 0.15) | 0.09 (0.01; 0.16)* | 0.02 (−0.06; 0.09) |
| Glucose (mmol/l)| −0.02 (−0.08; 0.07) | −0.04 (−0.11; 0.04) | 0.03 (−0.06; 0.09) |
| γGT (IU/l)      | −0.06 (−0.15; 0.02) | −0.03 (−0.11; 0.04) | 0.06 (−0.01; 0.14) |
| CRP (mg/l)      | −0.01 (−0.08; 0.07) | −0.19 (−0.26; −0.12)*** | −0.13 (−0.20; −0.06)*** |

Spearman rank correlation (95% CI). *P<0.05, **P<0.01, ***P<0.001.

binding studies, suggesting IF1 is not likely associated with HDL (20, 21). Whether IF1 is circulating as an intact and active protein will require further investigation. However, our previous data showed that IF1 could be immunoprecipitated from serum into two immunoreactive products of ~20 kDa each (14). Although this size could correspond to a dimer of IF1, which is the main active form of IF1 within the mitochondria (22), we cannot exclude that the immunoprecipitated products reflect some posttranslational modifications or alternative splicing of IF1 or that circulating IF1 is associated with other serum compounds.

The observed relationships between circulating IF1 and HDL levels lend support to the view that, in humans, this new F$_1$-ATPase pathway is physiologically relevant in HDL metabolism. At present, only hypotheses can be proposed to explain this reciprocal relationship. IF1 is a mitochondrial protein, able to block the ATP hydrolytic activity of mitochondrial ATP-synthase (23). Components of this enzymatic complex, and particularly of the F$_1$-ATPase catalytic domain, have been found present at the surface of various cells, including hepatocytes (24). We have shown that apoA-I binds to cell surface F$_1$-ATPase and stimulates its activity.
ATP hydrolyzing activity, triggering P2Y13 ADP-receptor activation and a signaling cascade, which further leads to the uptake by hepatocytes of both protein and lipid moieties of the HDL particle, a process called holo-HDL endocytosis (8). This endocytosis pathway for HDL is distinct from the so-called selective HDL cholesterol uptake mediated by the scavenger receptor B1 (SR-B1), a mechanism by which cholesterol is preferentially taken up, while the protein components of the HDL particle are not (25). Interestingly, addition of exogenous IF1 was able to reduce HDL uptake by cultured human hepatocytes and perfused rodent livers, most likely by inhibiting apoA-I-induced F1-ATPase activity (8). Thus, serum IF1 activity might slow down HDL hepatic catabolism, increasing the residence time of HDL in serum. IF1 concentrations were also negatively correlated to levels of TG-rich, apoB-containing lipoproteins. The inverse relationship between metabolism of HDL and TG-rich lipoproteins is well known, being mediated by lipid transfer proteins and lipoprotein lipase. Thus, as IF1 concentration is closely related to HDL-C, its inverse relation with TG markers might reflect the same metabolic interactions. However, in multivariate analysis, TGs were independent determinants of IF1. Thus we cannot exclude a direct impact of IF1, either on the metabolism of TG-rich lipoproteins or an impact on adipocytes, which control TG storage and mobilization (26). Interestingly, functional ecto-F1-ATPase has been described at the surface of adipocytes (27).

TABLE 4. Risk of coronary heart disease using HDL-C, apoA-I, and IF1 as explanatory variables

| Models with & Q1 | Q2 OR (95% CI) | Q3 OR (95% CI) | Q4 OR (95% CI) | P for Trend |
|------------------|---------------|---------------|---------------|------------|
| HDL-C            |               |               |               |            |
| 1                | 0.29 (0.19; 0.47) | 0.15 (0.09; 0.24) | 0.10 (0.06; 0.16) | 0.001 |
| ApoA-I           |               |               |               |            |
| 1                | 0.19 (0.12; 0.32) | 0.08 (0.05; 0.14) | 0.04 (0.02; 0.07) | 0.001 |
| IF1              |               |               |               |            |
| 1                | 0.67 (0.45; 1.02) | 0.38 (0.25; 0.58) | 0.20 (0.13; 0.32) | 0.001 |
| IF1 †           |               |               |               |            |
| 1                | 0.80 (0.52; 1.24) | 0.53 (0.34; 0.82) | 0.31 (0.19; 0.48) | 0.001 |
| IF1 ‡           |               |               |               |            |
| 1                | 0.84 (0.53; 1.32) | 0.69 (0.43; 1.09) | 0.41 (0.25; 0.65) | 0.001 |

All models adjusted on treatments for dyslipidemia, hypertension and diabetes; on smoking, physical activity, and alcohol consumption; and on CRP, Lp(a), and triglyceride. Quartiles for HDL-C: 0.36, 0.43, 0.51 g/l. Quartiles for apoA-I: 1.19, 1.37, 1.55 g/l. Quartiles for IF1: 0.382, 0.466, 0.560 mg/l.
† Plus HDL-C.
‡ Plus apoA-I.

TABLE 5. Concomitant risks of CHD for HDL-C, apoA-I, and IF1

| IF1 (mg/l) | OR (95% CI) |
|-----------|-------------|
| < 0.47    |             |
| ≥ 0.47    |             |
| HDL-C (g/l) |       |       |
| < 0.43    | 1           | 0.31 (0.20; 0.48) |
| ≥ 0.43    | 0.24 (0.15; 0.37) | 0.14 (0.09; 0.21) |
| ApoA-I (g/l) |     |     |
| < 1.37    | 1           | 0.32 (0.20; 0.51) |
| ≥ 1.37    | 0.14 (0.09; 0.21) | 0.10 (0.07; 0.15) |

Adjusted for dyslipidemia, hypertension, diabetes, smoking, physical activity, alcohol consumption, CRP, Lp(a), and triglyceride. IF1 × HDL-C interaction, P = 0.04. IF1 × apoA-I interaction, P = 0.009. Median cutoff values for IF1, HDL-C and apoA-I analyses were considered from both sides of median values.

by which cholesterol is preferentially taken up, while the protein components of the HDL particle are not (25). In-
the calcium-modulated protein calmodulin could sequester IF1 on the plasma membrane of hepatocytes, suggesting that the IF1 sorting pathway could depend on calcium signaling (29). Also, under acute cholestasis induced by short-term bile duct ligation in rat, the level of IF1 was found increased at the liver plasma membrane, indicating that the IF1-trafficking pathway might be regulated by hepatobiliary cholesterol metabolism (30). Moreover, mobilization of mitochondrial IF1 in cardiomyocytes in response to experimental ischemia has been previously reported (31, 32); hence, serum IF1 concentration might partly reflect a subtle balance between its utilization and release, depending on the cell energetic status. Finally, environmental factors, which are known to modulate HDL levels, had little or no influence on IF1 concentrations, suggesting that individual factors (genetic and metabolic) might control serum IF1 levels.

Statistically significant interactions were evidenced between IF1 and HDL-C or apoA-I concentrations regarding their association to CHD. Most particularly, in subjects with low HDL-C or apoA-I levels, a high IF1 was associated with a 70% reduction in the OR for CHD. The relative decrease was much less pronounced in subjects with elevated HDL. In mechanistic terms, this suggests that curbing HDL liver catabolism might be beneficial in patients who have low levels of circulating HDL. This raises the question of how depressing HDL catabolism might be beneficial against atherosclerosis. Although a major role of HDL is its central involvement in RCT, HDL particles by themselves exert pleiotropic effects on vascular cells, impairing LDL-oxidation and enhancing endothelial function, probably by downregulating endothelial expression of monocyte adhesion molecules, while promoting synthesis and bioavailability of nitric oxide (33, 34). It can thus be hypothesized that slowing down HDL catabolism would prolong residence time of HDL particles, enabling them to exert pleiotropic beneficial effects. This might not have a major impact on the whole process of RCT since, in humans, the final steps (liver uptake) occur not only through selective HDL-C uptake via SR-BI (25) or possibly holo-HDL endocytosis via ecto-F1-ATPase pathway (8) but also through LDL uptake following lipid transfers between lipoproteins (35). Interestingly, it has been shown that the catabolism of HDL particles is accelerated in diabetes, which leads to a significant reduction in HDL plasma residence time. This increased HDL turnover, potentially detrimental regarding HDL vascular effects, was corrected by rosuvastatin (36). Thus, IF1 and other molecules that slow down the hepatic catabolism of HDL, like SR-BI inhibitors (37, 38) or niacin (39), might exert beneficial effects through increasing the residence time of HDL particles in serum, prolonging their multiple effects on the vascular wall.

Of course the study design, a case-control comparison, limits our conclusions in several ways and no causal relationship can be established. Therefore, further measurements are foreseen within the framework of a large prospective cohort to establish more firmly the predictive value of IF1 determinations.

In conclusion, serum IF1 measurements may prove valuable in the assessment of CHD risk along with established risk factors, including HDL-C. Its diagnostic potential might be particularly important in people who have low levels of HDL-C. Further studies will be needed to explore its regulation in different pathophysiological contexts.

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