Fatty Liver Index, Gamma-Glutamyltransferase, and Early Carotid Plaques

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An association between fatty liver and carotid atherosclerosis has been established; however, it is not clear whether this relationship is a consequence of shared conventional risk factors or whether it is determined by specific circulating factors originating from liver or adipose tissue. To identify the factors possibly linking fatty liver and atherosclerosis, we assessed, in 1,012 subjects free of confounding diseases (e.g., hypertension, diabetes, cardiovascular diseases, and dyslipidemia) and metabolic syndrome, the relationship between the presence of early plaques at carotid bifurcation and fatty liver index (FLI; a validated surrogate marker of fatty liver), as well as the associations between carotid plaque presence and established atherosclerotic risk factors, family history of cardiovascular disease (FH-CVD) or diabetes, insulin sensitivity, serum liver enzymes, adipokines, fatty free acids, and high-sensitivity C-reactive protein (hsCRP). A total of 55 of 1,012 subjects (5.4%) had small plaque at carotid bifurcation. Subjects with plaque were older and had higher prevalence of FLI $\geq$60 and FH-CVD, higher blood pressure, plasma low-density lipoprotein cholesterol, glucose, gamma-glutamyltransferase (GGT), and hsCRP, as compared to subjects without plaques ($P < 0.05$). In a logistic regression model, adjusted for sex, liver transaminase, and alcohol consumption, the independent predictors of plaque presence were age ($P < 0.0005$), FLI $\geq$60 ($P < 0.0005$), and current smoking ($P < 0.05$). When FLI in the model was replaced by variables used in its equation (e.g., body mass index, waist circumference, plasma triglycerides, and GGT), the independent determinants of plaque presence were age ($P < 0.001$), GGT ($P = 0.001$), and current smoking ($P < 0.05$). *Conclusions:* Our cross-sectional study suggests that subjects with FLI $\geq$60 are at higher risk of atherosclerotic lesions, independently of established risk factors, and that serum GGT may represent a link between fatty liver and the development of early atherosclerosis. (HEPATOLOGY 2012;55:1406-1415)

Published studies have demonstrated an association between fatty liver and carotid atherosclerosis,[1,2] above all, carotid atherosclerotic plaques.[3] However, it is not clear whether the relationship between the fatty liver and atherosclerotic process is a consequence of shared established risk factors (e.g., abdominal obesity, atherogenic dyslipidemia, hypertension, and dysglycemia)[4,5] or whether it is determined by specific circulating factors that originate either from liver or from adipose tissue[6] and are known to participate in the development and progression of atherosclerosis through their effects on smooth muscle cell (SMC) proliferation and migration, foam cell formation, inflammation, and angiogenesis.[7–11]

To provide insight about the possible links between fatty liver and the development and progression of carotid atherosclerosis, we analyzed data from the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) Study that included a clinically healthy young-to-middle-aged European Caucasian population

Abbreviations: ATP III, National Cholesterol Education Program's Adult Treatment Panel III report; BMI, body mass index; BP, blood pressure; CCA, common carotid artery; CNR, Consiglio Nazionale delle Ricerche; CVDs, cardiovascular diseases; DELFIA, dissociation-enhanced lanthanide fluorescent immunoassay; FH-CVD, family history of cardiovascular disease; FLI, fatty liver index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HRT, hormone replacement therapy; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; LDL, low-density lipoprotein; MGL, mean gray level; MI, index of insulin sensitivity; NEFAs, nonesterified fatty acids; OGTT, oral glucose tolerance test; PA, physical activity; RISC, Relationship between Insulin Sensitivity and Cardiovascular risk Study; ROI, region of interest; SD, standard deviation; SE, standard error; SMCs, smooth muscle cells; US, ultrasound.

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that was free of confounding morbidities, such as hypertension, diabetes, dyslipidemia, and cardiovascular diseases (CVDs).[12] In such a selected population, we assessed the relationship between the presence of small plaque at carotid bifurcation and fatty liver, as well as the cross-sectional associations between carotid plaque presence and conventional atherosclerotic risk factors (e.g., sex, age, anthropometric parameters, blood pressure [BP], lipid profile, smoking habit, low physical activity [PA] level, and family history of CVD and diabetes), insulin resistance, and several circulating molecules, possibly proatherogenic and originating from liver or adipose tissue (e.g., liver enzymes, adipokines, fatty free acids, and high-sensitivity C-reactive protein; hsCRP). The presence of fatty liver was estimated by a validated surrogate marker, the fatty liver index (FLI).[13,14]

Small plaques at carotid bifurcation represent a good clinical model of early atherosclerosis, because carotid bifurcation is one of the first, and the most common, sites of atherosclerotic plaque formation resulting from interactions between local hemodynamic factors, local cellular factors, and active circulating molecules.[15,16] Besides the presence of early carotid plaques, we also assessed their acoustic properties by means of videodensitometric analysis, which has been previously shown to provide information on plaque composition, because it can identify plaque with high content of lipid or SMCs.[17,18]

Patients and Methods

The study population was a subgroup of the RISC study cohort (www.egir.org). The details of the study design and protocol have been reported elsewhere.[12] Briefly, RISC recruited apparently healthy Caucasian subjects in 19 centers in 14 European countries between June 2002 and July 2004. They were between 30 and 60 years of age with BP, serum cholesterol, triglycerides, fasting, and 2-hour glucose concentrations within established limits. Exclusion criteria were the presence of overt CVD, chronic diseases (e.g., hypertension, diabetes, dyslipidemia, chronic lung, hepatic and kidney diseases, and neoplastic and inflammatory diseases), class III obesity, the presence of carotid stenosis >40% and calcified carotid plaques, and treatment for hypertension, diabetes, dyslipidemia, obesity, and steroid treatment (with the exception of hormone replacement therapy [HRT] in menopausal women). A standardized examination protocol included anthropometry, brachial BP measurements, resting electrocardiogram, a fasting blood test, an oral glucose tolerance test (OGTT), a euglycemic hyperinsulinemic clamp, and high-resolution ultrasound (US) of extracranial carotid arteries. Information regarding medical history, drug use, alcohol and cigarette consumption, and family history (i.e., any first-degree family member) of CVD (FH-CVD) (i.e., coronary heart disease and stroke) and diabetes were collected using standardized self-reported questionnaires. For smoking habit, the subjects were categorized as never smoker, current smoker, and ex-smoker (when quitted smoking for 1 year and more before the study). Data on alcohol consumption were not rechecked with family members. Metabolic syndrome was defined according to the criteria of the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III), which have been suggested to provide a practical tool for identification of subjects at increased risk of CVD.[19] The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the local ethics committee in each center. Written consent was obtained from all participants.

Population of the Present Study. In the RISC study, 1,566 participants were originally recruited; 356 were excluded for not satisfying inclusion criteria or for incomplete baseline examination (Fig. 1). For the purpose of this study, we also excluded subjects in whom the carotid bifurcation and origin of internal carotid artery could not be adequately visualized as well as subjects with an above-average or a high relative Framingham risk score[20] and with metabolic syndrome according to ATP III criteria.[19] The final study population consisted of 1,012 apparently healthy subjects with complete baseline data, adequate visualization of the entire extracranial carotid tree, at a low-average relative Framingham risk, and free of metabolic syndrome. In a subpopulation of 669 subjects (66.1% of the population of the present study), an objective assessment of habitual PA by means of accelerometer monitoring[21] was also available (Fig. 1).

Body Composition Assessment and BP Measurement. Body weight and fat-free mass were measured by electrical bioimpedance using a Body Composition

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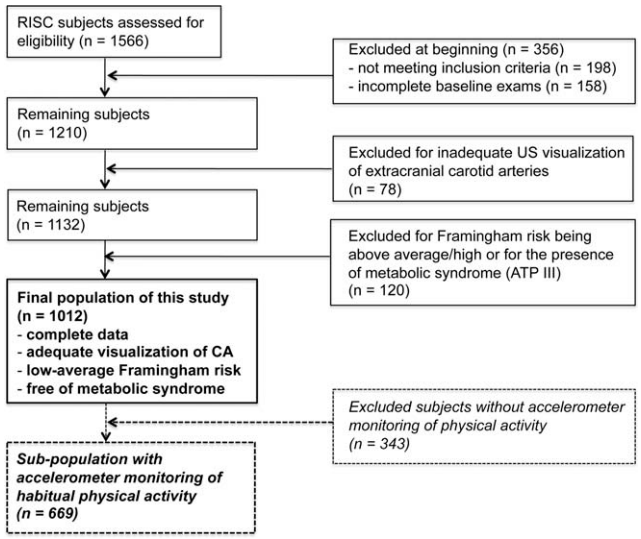

Fig. 1. Selection of the present study population from the RISC population.

Analyzer (model TB-300; Tanita, Tokyo, Japan); fat mass was obtained as the difference between body weight and fat-free mass. Waist circumference was measured as the narrowest circumference between the lower rib margin and anterior superior iliac crest. Brachial BP was measured by a digital electronic tensiometer (model 705cp; Omron, Kyoto, Japan), with regular or large adult cuffs according to the arm circumference, in subjects seated for at least 10 minutes.

OGTT and Insulin Clamp. A 75-g OGTT was performed, with blood samples taken before and 30, 60, 90, and 120 minutes into the test. On a separate day within 1 month of the OGTT, a euglycemic hyperinsulinemic clamp was performed. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 $pmol \cdot min^{-1} \cdot m^{-2}$ simultaneously with a variable 20% dextrose infusion adjusted every 5-10 minutes to maintain a plasma glucose level within 0.8 mmol/L (± 15%) of the target glucose level (4.5-5.5 mmol/L). Additional blood samples were obtained at 20-minute intervals for insulin determination. The clamp procedure was standardized across centers.[12,22] Insulin sensitivity was expressed as the ratio of the M value averaged over the final 40 minutes of the 2-hour clamp and normalized by the fat-free mass, to the mean plasma insulin concentration measured during the same interval (M/I; in units of $\mu mol \cdot min^{-1} \cdot kg_{ffm}{}^{-1} \cdot mm^{-1}$).[22]

Analytical Procedures. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (Roche Method for Modular System; Roche Diagnostics, Basel, Switzerland), glucose (Cobas Integra; Roche Diagnostics), insulin (a specific time-resolved fluoroimmunoassay, AutoDELFIA [dissociation-enhanced lanthanide fluorescent immunoassay] Insulin kit; Wallac Oy, Turku, Finland), liver enzymes (International Federation of Clinical Chemistry method; Dade-Behring Dimension RXL, Newark, DE), leptin (an in-house DELFIA assay on an AutoDELFIA autoanalyzer; Wallac Oy), total plasma adiponectin (in-house time-resolved immunofluometric assay[23]), nonesterified fatty acids (NEFAs; Randox enzymatic kit, Hitachi Modular P unit; Hitachi, Tokyo, Japan), and hsCRP (monoclonal antibodies from R&D Systems; Abingdon, UK) were measured centrally.

Surrogate Marker of Fatty Liver. A surrogate marker of fatty liver, the FLI, was calculated.[13,14] An FLI that uses an algorithm based on body mass index (BMI), waist circumference, triglycerides, and gamma-glutamyltransferase (GGT) has been validated against liver US in the general population and has been proven accurate in detecting fatty liver (accuracy, 0.84 [95% confidence interval: 0.81-0.87]).[13] When the index value is greater than or equal to 60 (FLI $\geq$60), the probability of having a fatty liver is >78%.[14] A validation of FLI against magnetic resonance spectroscopy demonstrated the presence of hepatic fat (range, 8.6%-24.0%) in subjects with FLI $\geq$60 and the absence of hepatic fat in those with FLI <20.[24]

Physical Activity Assessment. Habitual PA was estimated by accelerometer monitoring. A single-axis accelerometer (Computer Science Applications Model AM7164; Manufacturing Technology, Inc., South Bend, IN) was used to monitor ambulatory movements.[21] The accelerometer was secured by a belt at the small of the back from waking up until going to sleep. Subjects were asked to wear the monitor for 7 days, if possible, weekend included, and to behave in their usual manner. In the final analysis, only those days when the accelerometer was worn for at least 10 hours were included. Nonwearing periods were identified as 60 minutes or more of continuous zero counts. Accelerometer data were processed with custom software developed for the RISC project and were checked for spurious recording: high counts >20,000 counts/min or repeated recording of the same number of counts[25]; the days with spurious data were excluded. The average intensity of daily PA was expressed as the average number of accelerometer counts per 1 minute of monitoring time.

Carotid Artery US Imaging and Analysis. High-resolution B-mode US of extracranial carotid arteries was performed in each recruiting center by trained and certified technicians following a standardized

protocol.[12] Reading of carotid images was centralized at one center (University of Pisa, Pisa, Italy) by a single reader (M.K.) using the Medical Image Processing computer-driven image analysis system (Institute of Clinical Physiology, Consiglio Nazionale delle Ricerche [CNR], Pisa, Italy). Near- and far-wall intima-media thickness (IMT) was measured bilaterally in digitized end-diastolic frames for a single view of the common carotid artery (CCA) and for three different views of the carotid bulb and internal carotid artery. Carotid plaque was defined as an IMT >1.5 mm in any carotid segment.[26] CCA IMT represents a mean value of far-wall IMT measured in the right and left artery. In the RISC study, intraobserver variability of IMT measurements was tested in 140 randomly chosen scans. The correlation between two readings was r = 0.95 ($P < 0.0001$), and the mean difference was 4.8%. ± 2.8%.

Acoustic Videodensitometric Analysis of Carotid Plaques. The acoustic properties of the carotid plaque were evaluated by means of digital densitometric analysis (Institute of Clinical Physiology, CNR, Pisa, Italy) that was previously validated against histological analysis of intimal lesions.[18] In the long-axis view, a region of interest (ROI) including the entire carotid plaque was selected. Within the ROI, digitized images were analyzed by the first-order analysis that generates a histogram representing the frequency distribution of gray levels of pixels by plotting the gray values on the abscissa and the frequency of the occurrence on the ordinate. The histogram was described in terms of average pixel intensity, that is, mean gray level (MGL). To adjust for different gain settings and different US attenuation in different study subjects, two calibration steps were introduced into the analysis of each subject. The effect-of-gain setting was restrained by calibrating the gray-level amplitude of the ROI against vessel lumen (i.e., blood) taken as the blank (MGL = 0), whereas the effects of imaging depth and attenuation were minimized by calibration against an internal reference represented by the adventitia (MGL = 160).[18] Intraobserver variability of MGL measurement was tested in 30 randomly chosen plaques. The correlation between two readings was r = 0.93 ($P < 0.0001$), and the corresponding mean difference was 6.7% ± 5.0%.

Statistical Analysis. Quantitative data are expressed as mean ± standard deviation (SD), and categorical data are expressed as percentages. Skewed variables are given as median and interquartile range and were log-transformed for statistical analyses. Analysis of variance was used to compare continuous variables. Relations between the outcome variables and continuous variables were evaluated by univariate Pearson's correlation coefficients. Logistic regression analysis and multiple linear regression analysis adjusted for center were used to test the independence of the associations of outcome variables with their significant correlates in univariate models. Statistical tests were two-sided, and significance was set at a value of $P < 0.05$. Statistical analysis was performed by JMP software (version 3.1; SAS Institute, Inc., Cary, NC).

Table 1. Characteristics of Subjects Without and With Early Carotid Plaque

	Without Plaque	With Plaque	*P* Value
N	957	55	
FLI	26 ± 23	40 ± 28	<0.0001
FLI ≥60 (%)	11.1	36.3	<0.0001
Sex (men; %)	42.4	50.9	=0.22
Age (years)	43 ± 8	49 ± 8	<0.0001
Waist circumference (cm)	85 ± 12	87 ± 13	=0.12
BMI (kg/m^2)	24.9 ± 3.7	25.6 ± 3.4	=0.14
Fat mass (kg)	19.8 ± 8.1	20.7 ± 7.5	=0.44
Systolic BP (mmHg)	116 ± 12	121 ± 12	=0.01
Diastolic BP (mmHg)	74 ± 8	77 ± 7	=0.01
Heart rate (bpm)	68 ± 11	66 ± 9	=0.32
LDL cholesterol (mmol/L)	2.9 ± 0.8	3.2 ± 0.7	<0.001
HDL cholesterol (mmol/L)	1.46 ± 0.38	1.44 ± 0.35	=0.61
*Triglycerides (mmol/L)	0.9 [0.5]	1.1 [0.6]	=0.17
Fasting plasma glucose (mmol/L)	5.0 ± 0.6	5.2 ± 0.5	=0.01
*Fasting plasma insulin (pmol/L)	29 [20]	35 [23]	=0.32
*M/I (μmol.min^{-1}·kg$_{FFM}$$^{-1}$·nmol/L^{-1})	137 [89]	130 [67]	=0.53
*ALAT (IU/L)	17 [10]	17 [17]	=0.12
*ASAT (IU/L)	20 [7]	22 [8]	=0.15
*GGT (IU/L)	20 [12]	27 [21]	<0.0001
*Alcohol consumption (g/week)	42 [82]	42 [76]	=0.52
*Adiponectin (mg/L)	8.3 [4.1]	7.9 [4.5]	=0.98
*Leptin (ng/ml)	8.7 [12.3]	10.3 [9.0]	=0.61
*NEFA (mmol/L)	0.50 [0.27]	0.47 [0.31]	=0.18
*hsCRP (mg/L)	0.4 [0.8]	0.8 [1.2]	<0.05
Smoking habit (never:ex:current; %)	52:24:24	44:25:31	=0.43
FH-CVD (%)	33.1	49.1	=0.01
FH of diabetes mellitus (%)	23.8	27.7	=0.89
†Average daily PA (counts per minute)	377 ± 177	304 ± 141	<0.01

Abbreviations: FH, family history; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

*Skewed variables expressed as median [interquartile range].

†Data on PA available only in 669 subjects.

Results

Of 1,012 apparently healthy young-to-middle-aged subjects, 55 (5.4%) had 1 or 2 small atherosclerotic plaques without calcification at the carotid bulb and/or origin of internal carotid artery (overall, 68 plaques; maximum plaque thickness = 2.06 ± 0.38 mm). Subjects with plaques had higher FLI, higher prevalence of FLI ≥60, were older, had higher office BP, plasma level of LDL cholesterol, fasting glucose, GGT, and hsCRP, and a higher prevalence of CVD within first-degree relatives (Table 1), as compared to subjects

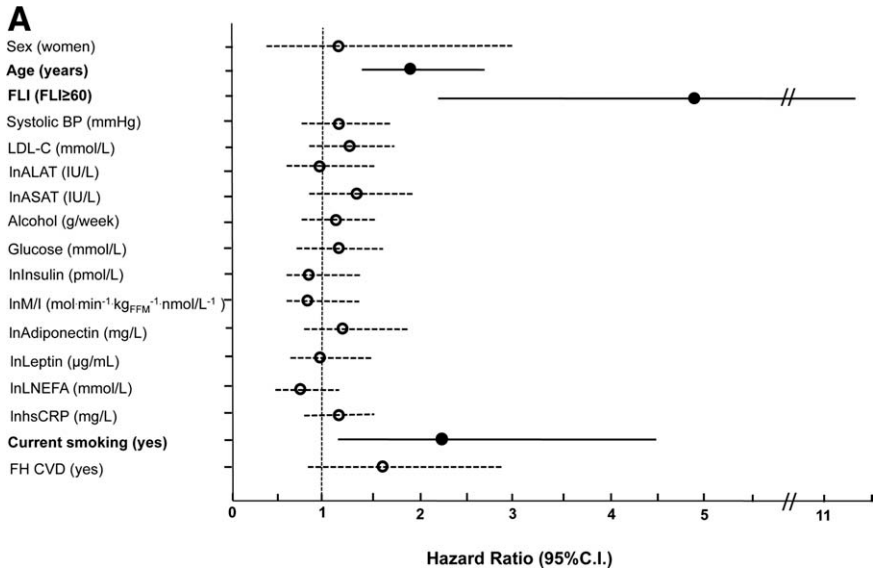

Fig. 2A. Independent predictors (full circles and lines) of the presence of early carotid plaque. Logistic regression model including FLI, used as a dichotomic variable (FLI $\geq$60 and FLI$<$60). Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln).ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

without plaques. Subjects with and without plaques were comparable for sex distribution, anthropometric parameters, liver transaminase and alcohol consumption, fasting insulin and insulin sensitivity, plasma levels of adiponectin, leptin and NEFA, and prevalence of diabetes within the first-degree relatives. Smoking habit also did not differ significantly between subjects with and without plaque (Table 1). However, within current smokers (N = 246; mean duration of smoking, 22 $\pm$ 9 years; mean cigarette consumption, 15 $\pm$ 10 cigarettes per day), those with plaque (N = 17) had smoked for a longer period, as compared to those without plaques (29 $\pm$ 10 versus 22 $\pm$ 8 years; $P <$ 0.01); average cigarette consumption was comparable (15 $\pm$ 7 versus 14 $\pm$ 11 cigarettes per day; $P = 0.81$) between the two groups. One hundred and three women used HRT; the prevalence of FLI $\geq$60 was comparable between women with and without HRT (4.9% versus 6.5%; $P = 0.52$).

In a subgroup of 669 subjects (276 men and 393 women) undergoing accelerometer monitoring of PA, the mean monitoring time was 5.6 $\pm$ 1.5 days. Subjects with small carotid plaques (N = 46) had lower average daily PA, as compared to those without plaques (Table 1).

To assess whether the association between hepatic steatosis and early carotid atherosclerosis was independent of common risk factors, a logistic regression analysis was used, entering as a dependent variable the presence of carotid plaque and as independent variables the index of hepatic steatosis (as a dichotomic variable, FLI $\geq$60 and FLI $<$60), together with possible determinants of atherosclerotic process (e.g., sex, age, systolic BP, lipid profile, glycemia, insulin and insulin sensitivity, adipokines, NEFA, and hsCRP; normalized for 1 SD) (Fig. 2A). The analysis was adjusted for center, current smoking, alcohol consumption, liver transaminase, and FH-CVD. In such a model, the independent determinants of carotid plaque presence were age ($P < 0.0005$), FLI $\geq$60 ($P < 0.0005$), and current smoking ($P < 0.05$). Subsequently, the FLI was replaced in a logistic regression model by variables used in its equation (e.g., BMI, waist circumference, plasma triglycerides, and GGT) to identify the possible pathophysiologic link between fatty liver and early carotid atherosclerosis. In this model (Fig. 2B), the independent determinants of plaque presence were age ($P < 0.001$), GGT ($P = 0.001$), and current smoking ($P < 0.05$). Finally, the same logistic analysis was run only in 967 subjects with plasma GGT within normal limits (up to 40 IU/L in women and up to 68 IU/L in men).[27] Independent determinants of carotid plaque presence in this model were age ($P < 0.0005$) and current smoking ($P = 0.01$), but not GGT ($P =$ 0.14).

When average daily PA was added into the logistic regression model in a subpopulation of 669 subjects with accelerometer monitoring, it was entered as an additional independent predictor of plaque presence without affecting the other determinants (Fig. 2C).

CCA IMT was also higher in subjects with FLI $\geq$60, as compared to those with FLI $<$60 (677 $\pm$ 97 versus 590 $\pm$ 79 μm; $P < 0.01$, after adjustment for age). In the entire study population, CCA IMT correlated with age (r = 0.42; $P < 0.0001$), anthropometric parameters (r = 0.17-0.28; $P < 0.0001$ for all),

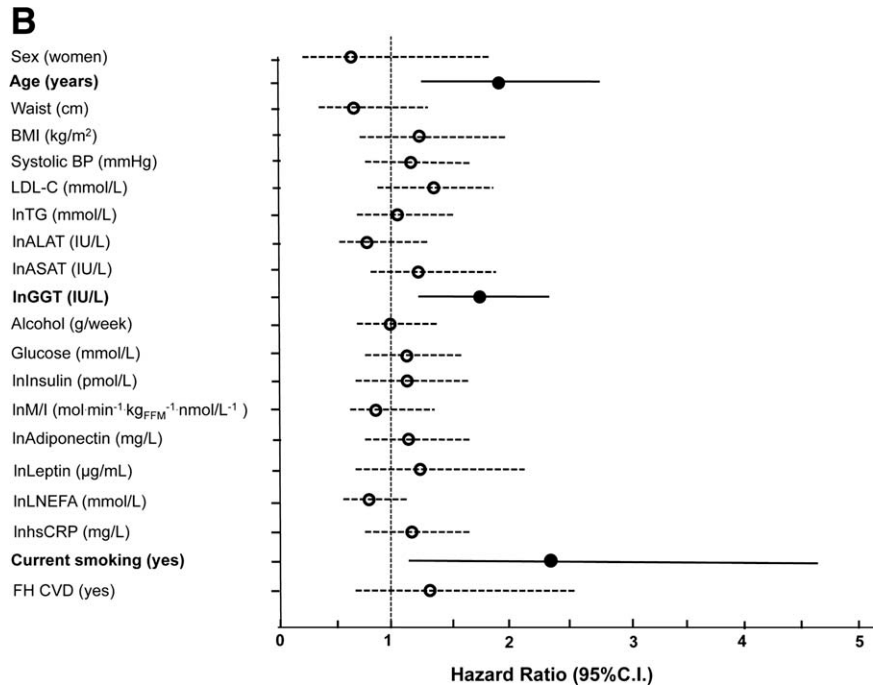

Fig. 2B. Independent predictors (full circles and lines) of the presence of early carotid plaque. Logistic regression model including variables used in FLI equation (BMI, waist circumference, plasma triglycerides, and GGT). Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln). ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

systolic BP (r = 0.27; $P < 0.0001$), plasma LDL cholesterol and triglycerides (r = 0.26 and 0.21; $P < 0.0001$ for all), fasting plasma glucose (r = 0.17; $P < 0.0001$), and plasma GGT (r = 0.16; $P < 0.0001$). A multivariate regression model was created, entering as a dependent variable the standardized CCA IMT and as independent variables FLI (as a dichotomic variable) and all the variables that correlated with CCA IMT in a univariate model. The model was adjusted for center, sex, current smoking, alcohol consumption, and family history. Following backward stepwise removal, the independent predictors of CCA IMT were male sex, age, FLI $\geq$60, systolic BP, LDL cholesterol, and FH-CVD (Table 2). When FLI in the model was replaced by the variables used in its calculation, independent predictors of CCA IMT were male sex, age, waist circumference, systolic BP, LDL cholesterol, and FH-CVD.

Tissue Characterization of Early Carotid Plaques. Densitometric analysis was performed in 52 plaques (excluding plaques not entirely visualized and, in subjects with 2 plaques, the smaller one). MGL of carotid plaques was higher in subjects with FLI $\geq$60, as compared to those with FLI <60 (Fig. 3A), and in current smokers, as compared to nonsmokers and ex-smokers (81 $\pm$ 26 versus 64 $\pm$ 22; $P < 0.05$). Plaque MGL increased with waist circumference, 2-hour plasma glucose, and insulin (r = 0.32, 0.35, and 0.31; $P < 0.05$ for all) and decreased with plasma adiponectin concentrations (Fig. 3B). In the multivariate regression model (adjusted for center, sex, and smoking habit), plasma adiponectin and current smoking were the only independent determinants of plaque MGL ($\beta \pm$ standard error [SE] = -0.42 ± 0.11, $P < 0.001$, and 0.26 ± 0.11, $P < 0.05$; cumulative $R^2 = 0.55$, $P < 0.0001$). In addition, subjects with FLI $\geq$60 had lower plasma levels of adiponectin (Fig. 3C) and plasma adiponectin was inversely related to FLI (Fig. 3D). Also, in the entire study population (N = 1,012), plasma adiponectin levels were lower in the subgroup with FLI $\geq$60, as compared to that with FLI <60 (6.8 ± 2.6 versus 9.0 ± 3.6; $P < 0.0001$) and plasma adiponectin and FLI were inversely correlated (r = -0.37; $P < 0.0001$).

Discussion

This cross-sectional study demonstrates that in apparently healthy young-to-middle-aged subjects without metabolic syndrome and increased cardiovascular risk, the presence of early plaques at the carotid bifurcation is independently associated with FLI $\geq$60, which is used as a surrogate marker of hepatic steatosis. When FLI was replaced by variables used in its equation (e.g., BMI, waist circumference, plasma triglycerides, and GGT), plasma levels of GGT emerged as an independent determinant of early carotid atherosclerosis. The association between GGT and carotid plaques was independent of established atherosclerotic risk factors, insulin sensitivity, adipokines, hsCRP, and PA level, but disappeared when only subjects within the normal GGT range were included into the analysis. These findings suggest that plasma GGT might

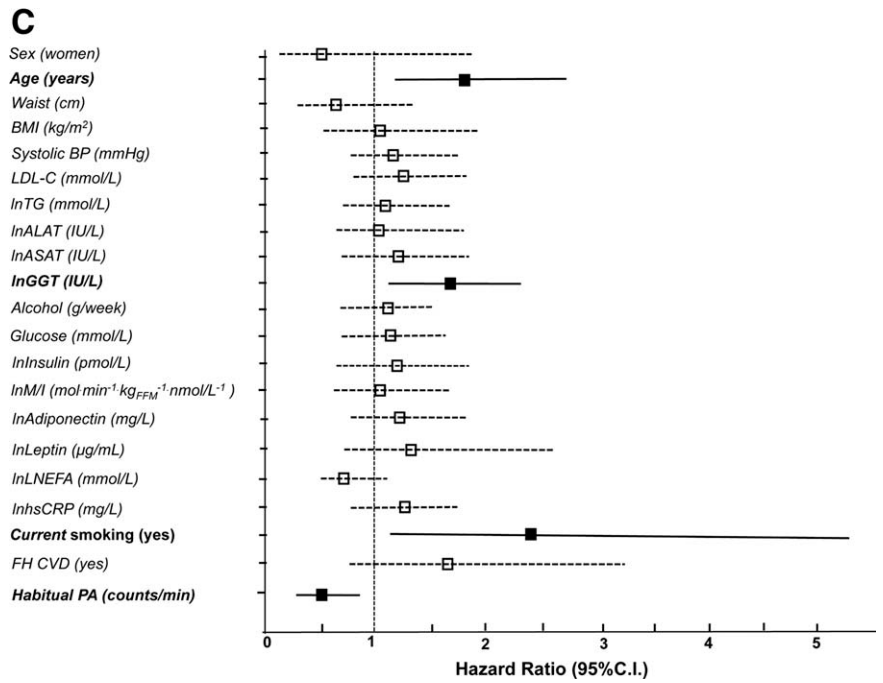

Fig. 2C. Independent predictors (full squares and lines) of the presence of early carotid plaque in a subpopulation of 669 subjects with accelerometer monitoring of habitual PA. Hazard ratios are calculated for 1 SD of the continuous variables. Skewed variables are log-transformed (ln). ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

represent a link between hepatic steatosis and the atherosclerotic process, and that GGT above normal limits could be used as a biochemical marker of atherosclerosis.

Our clinical data on GGT are supported by experimental studies, in which a catalytically active GGT has been found within cerebral, carotid, and coronary plaques, when colocalized with oxidized LDLs and cluster of differentiation 68^+ foam cells.[8] Plaque GGT is supposed to derive from the plasma, in the form of complexes with LDL.[7] Once accumulated in the plaque environment, GGT retains its activity and triggers an iron-dependent oxidation of LDL in the extracellular space.[28] It is worth noting that in our population, plasma GGT was an independent determinant of plaque presence in the carotid bulb, but not of the arterial wall thickness in the common carotid artery. Such a difference might indicate that local mechanical forces resulting from flow-separation and flow-profile changes in the carotid bulb are necessary to promote the influx and accumulation of plasma-derived GGT within the arterial wall.[15,16]

The initialization and evolution of atherosclerotic plaque are closely linked with the presence of SMCs; intimal SMCs are the first cells present in adaptive intimal thickening.[16] The presence of SMCs within atherosclerotic plaques has been shown to influence their acoustic properties, which can be quantitatively evaluated by means of acoustic densitometry. We have previously demonstrated, using the same densitometric analysis as in this study,[18] that with increasing SMC content, MGL (or acoustic reflectivity) of the initial atherosclerotic lesion increases. In the present study, MGL of small carotid plaques was inversely and independently related to plasma adiponectin levels, an observation indicating that increasing circulating adiponectin decreases the SMC content of these early lesions. Furthermore, subjects with FLI $\geq$60 had a

Table 2. Independent Correlates of CCA IMT: Multiple Regression Model

Model with FLI (Used As a Dichotomic Variable; FLI $\geq$60 and FLI $<$60)		
	β* $\pm$ SE	*P* Value
Sex (male)	0.11 $\pm$ 0.03	$<$0.0005
Age (years)	0.37 $\pm$ 0.03	$<$0.0001
FLI $\geq$60	0.15 $\pm$ 0.04	$<$0.0005
Systolic BP (mmHg)	0.14 $\pm$ 0.03	$<$0.0001
LDL cholesterol (mmol/L)	0.09 $\pm$ 0.03	$<$0.005
FH-CVD (yes)	0.07 $\pm$ 0.03	$<$0.05
Cumulative R^2	0.28	$<$0.0001

Model With the Variables Used in FLI Calculation (BMI, Waist Circumference, Triglycerides, and GGT)		
	$\beta \pm$ SE	*P* value
Sex (male)	0.08 $\pm$ 0.03	$=$0.01
Age (years)	0.36 $\pm$ 0.03	$<$0.0001
Waist circumference (cm)	0.11 $\pm$ 0.03	$=$0.001
Systolic BP (mmHg)	0.13 $\pm$ 0.03	$<$0.0001
LDL cholesterol (mmol/L)	0.09 $\pm$ 0.03	$<$0.005
FH-CVD (yes)	0.06 $\pm$ 0.03	$=$0.05
Cumulative R^2	0.27	$<$0.0001

*β = standardized regression coefficient.

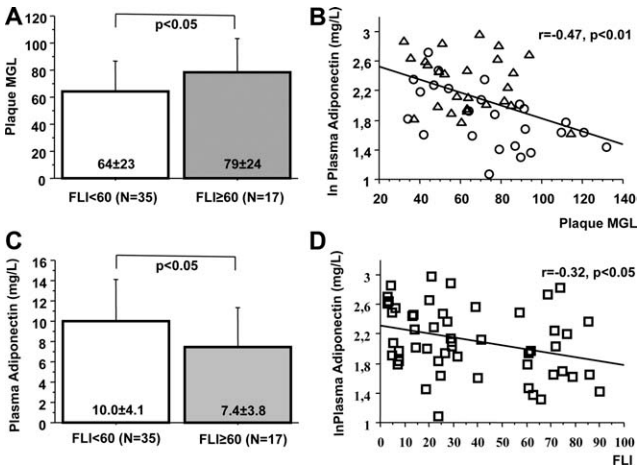

Fig. 3. (A) Plaque MGL in subjects with FLI <60 and FLI ≥60. (B) Correlation between plaque MGL and plasma adiponectin level. Circles indicate men, triangles indicate women. (C) Plasma adiponectin levels in subjects with FLI <60 and FLI ≥60. (D) Correlation between FLI and plasma adiponectin level in subjects with carotid plaques.

higher plaque MGL and a lower plasma adiponectin level, as compared to those with FLI <60, and plasma adiponectin and FLI were inversely related (Fig. 3). Altogether, these findings imply that the fatty-liver–related decrease in plasma adiponectin level may induce an increase in SMCs in early atherosclerotic plaques. Our observations are in agreement with *in vitro* studies demonstrating that adiponectin suppresses the platelet-derived growth factor–induced proliferation and migration of human aortic SMCs[10,29] and prevents adventitial fibroblasts from proliferating, transforming into myofibroblast, and migrating to the intima,[30] and they also confirm the previous clinical data on decreased plasma adiponectin levels in patients with fatty liver disease.[31] Yet, it must be emphasized that the observed effect of plasma adiponectin on the acoustic properties of carotid plaque might only apply to early atherosclerotic lesions in healthy subjects free of confounding pathologies and not to more advanced atherosclerotic plaques. The Prospective Investigation of the Vasculature in Uppsala Seniors Study, which includes the community-based elderly population with a high prevalence of hypertension, diabetes, and dyslipidemia, has shown a positive association between plaque MGL and plasma adiponectin levels that probably reflects the inhibiting effect of adiponectin on lipid accumulation and foam cell formation.[9,32]

Two additional findings should be briefly discussed. Cigarette smoking is supposed to accelerate the development and/or progression of carotid atherosclerosis,[33] and one of the mechanisms that may participate in early plaque formation in smokers is the effect of nicotine on SMCs. Nicotine induces SMC proliferation through the mediation of growth factors and inhibits physiological SMC apoptosis.[34] In line with these findings are our observations on associations between current smoking and smoking duration on one side and the presence and acoustic reflectivity of small carotid plaques on the other. Second, our data confirm the beneficial impact of PA on the atherosclerotic process that have been previously demonstrated in a variety of clinical and experimental studies and that is supposed to reflect the effect of PA on oxidative stress as well as pro- and anti-inflammatory cytokines.[35]

Strengths and Limitations. Our study includes a large population free of confounding diseases (e.g., CVD, diabetes, and hypertension), without metabolic syndrome, and at low-average cardiovascular risk. All subjects were well characterized from the metabolic point of view, including M/I by the gold-standard euglycemic hyperinsulinemic clamp. However, there were also some limitations. First, the design of the RISC study did not include liver US for the determination of fatty liver. Therefore, for the purpose of the present study, we used a previously validated surrogate marker, FLI,[13,14] that, in a recent prospective study, has been associated with cardiovascular mortality.[36] We also took into consideration the FLI developed by Kotronen et al.[37] However, the algorithm was not suitable for this population because it was developed in a group of subjects with diabetes and metabolic syndrome, whereas we studied clinically healthy subjects free of diabetes, hypertension, and metabolic syndrome. Second, because of the large number of centers participating in the RISC study, only standard US scanners were used for carotid imaging; thus, tissue characterization of carotid plaques was performed by densitometric analysis of B-mode images and not by analysis of raw radiofrequency data. Third, after a 3-year period, follow-up carotid US was available only in 35 subjects with carotid plaques, and consequently, follow-up data were not included into this study. Finally, information regarding family history of CVD, stroke, and diabetes as well as data on alcohol consumption and smoking habit were obtained by self-reported questionnaires and were not verified.

The results of this study indicate that in a healthy young-to-middle-aged population without metabolic syndrome and increased cardiovascular risk, plasma GGT may represent a pathophysiologic link between hepatic steatosis and the early atherosclerotic process, and that increased serum GGT might be used as a biomarker of atherosclerosis. Hepatic steatosis seems also to influence the acoustic properties of early carotid lesions, probably through its effect on plasma adiponectin level.

Appendix

RISC recruiting centers:
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