Clinical Biochemistry 45 (2012) 96–100

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

# The logarithm of the triglyceride/HDL-cholesterol ratio is related to the history of cardiovascular disease in patients with familial hypercholesterolemia

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ARTICLE INFO

*Article history:*
Received 2 May 2011
Received in revised form 2 November 2011
Accepted 8 November 2011
Available online 18 November 2011

*Keywords:*
Cardiovascular risk
Triglycerides/HDL-cholesterol ratio
Atherogenic index of plasma
Lipoprotein particle size

ABSTRACT

**Objectives:** The aim of this study was to determine whether the atherogenic index of plasma (AIP = log [triglycerides/HDL-cholesterol]) differs in heterozygous familial hypercholesterolemia (FH) patients with and without a history of cardiovascular disease (CVD).

**Design and methods:** A total of 555 FH patients with known mutations in the *LDL receptor* or the *apolipoprotein B* gene, of whom 53 had a history of CVD (CVD+ group), were retrospectively analyzed.

**Results:** Compared to patients without CVD (CVD− group), CVD+ patients showed significantly higher fasting LDL-cholesterol, triglycerides and AIP as well as lower HDL-cholesterol. After both adjustment for age and diabetes and using analysis based on age and sex matched groups, only the increase in triglycerides and AIP in the CVD+ vs. the CVD− group remained significant.

**Conclusion:** The results of the present study indicate that AIP, which reflects the presence of atherogenic small LDL and small HDL particles, may be connected to the risk of CVD in FH patients.



## Introduction

Elevated low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) are important risk factors for cardiovascular diseases (CVD), particularly for coronary artery disease (CAD) [1,2]. The size of lipoprotein particles also plays an important role in the atherogenic process. Small, dense LDL particles have been associated with CAD in a number of studies [3–5]. They possess a lower binding affinity for cellular LDL receptors and are more easily oxidized compared with large LDL particles, which suggests that small LDL particles are much more atherogenic [6–8]. Smaller LDL particles are predominant in a high proportion of CAD patients with normal LDL-C levels [9]. In addition, HDL particles are known to be heterogeneous in the population regarding their size, density and physicochemical properties [10,11]. Particularly large and buoyant HDL particles ($HDL_2$) play a protective role against the development of atherosclerosis, whereas the effect of small, dense HDL ($HDL_3$) particles on CVD risk remains controversial [12–15]. However, there is some evidence that small, dense $HDL_3$ particles are associated with an increased CVD risk [16–18]. LDL and HDL particle sizes are related to plasma levels of triglycerides (TG) [9,19]. The logarithm of the ratio TG/HDL-C correlates well with the size of HDL and LDL particles and with the fractional esterification rate of cholesterol by lecithin:cholesterol acyltransferase (LCAT) in plasma [20–22]. The fractional esterification rate of cholesterol in plasma depleted of apoB-containing lipoproteins (FER[HDL]) reflects the reactivity of HDL to LCAT and shows a strong positive correlation with plasma levels of small $HDL_{3b,c}$ particles and a strong negative correlation with the level of large $HDL_{2b}$ particles [23,24]. Thus, FER(HDL) is the fastest in the smallest HDL particles, is slower in larger HDL particles [25–27] and, together with LCAT activity, correlates with coronary atherosclerosis [22,28]. It has been established that the molar ratio of the concentration of TG/HDL-C is significantly increased in patients who have experienced a myocardial

*Abbreviations:* AIP, atherogenic index of plasma; APOB, apolipoprotein B gene; BMI, body-mass index; BP, blood pressure; CAD, coronary artery disease; CETP, cholesteryl-ester transfer protein; CVD, cardiovascular diseases; DM, diabetes mellitus; FER(HDL), fractional esterification rate of cholesterol; FH, familial hypercholesterolemia; HDL, high density lipoproteins; HDL-C, HDL-cholesterol; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LDL-C, LDL-cholesterol; LDLR, LDL receptor; TC, total cholesterol; TG, triglycerides.

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doi:10.1016/j.clinbiochem.2011.11.001

infarction, compared with age- and sex-matched control subjects [29]. The measurement of the FER(HDL) or particle sizes (LDL and HDL) is difficult to put into clinical practice, but it can be easily substituted by calculating the atherogenic index of plasma (AIP) [30]. It has been repeatedly shown that the AIP value (log[TG/HDL-C]) strongly correlates with FER(HDL) and with the size of the lipoprotein particles [21,22]. Thus, the AIP value accurately reflects the presence of atherogenic small LDL particles and small HDL particles, and it is also a sensitive predictor of coronary atherosclerosis and cardiovascular risk [22,28,31,32].

Familial hypercholesterolemia (FH) is an autosomal dominant disorder that is characterized by elevated LDL-C and premature atherosclerosis [33]. Previous FH studies have demonstrated smaller and denser HDL and LDL particles in patients with heterozygous FH compared to healthy controls; a negative correlation between cholesteryl-ester transfer protein (CETP) plasma levels and HDL and LDL particle size in FH patients; and a trend toward an increased history of CVD and increased IMT in the highest CETP concentration quartile compared to the other CETP concentration quartiles in FH patients [34–36]. However, no study has yet investigated whether a relationship exists between AIP and cardiovascular events in FH. The aim of this study was therefore to establish whether AIP differs among heterozygous FH patients with and without a history of CVD.

## Materials and methods

### *Study design and study population*

This was a retrospective, multicenter cohort study. We have maintained a MedPed (Make Early Diagnoses to Prevent Early Deaths in medical pedigrees) national project database of FH subjects in the Czech Republic, with the patients' demographics and clinical and laboratory data. To acquire the study population, the database was queried for patients with documented mutations in the *LDL-receptor* gene (LDLR group) or mutation in the *apolipoprotein B* gene (APOB group), who were at the time of entry into the database not undergoing hypolipidemic therapy and were at least 18 years old. This search returned 555 patients (378 unrelated and 177 related individuals; ratio 1.47 patients per affected family), of whom 53 had a history of CVD before entry into the database. CVD was defined by the presence of at least one of the following: a) coronary heart disease (myocardial infarction, angina pectoris, coronary artery bypass grafting or percutaneous coronary intervention); b) ischemic stroke or transient ischemic attack; or c) peripheral artery disease (peripheral arterial bypass graft or peripheral percutaneous transluminal angioplasty). Age, sex, body-mass index (BMI), systolic and diastolic blood pressure (BP), cigarette-smoking habits and the presence of diabetes mellitus (DM) were recorded.

### *Laboratory analysis*

The results of the laboratory analysis were acquired from our patient databases; lipid analyses were performed in local certified laboratories with appropriate quality control, using the identical analytic methods. Serum total cholesterol (TC) and TG levels were measured using fully automated enzymatic methods, and HDL-C was determined with the same method after the precipitation of apolipoprotein B-containing lipoproteins with polyanions. LDL-C was calculated using the Friedewald formula, but only if TG levels were <4.5 mmol/L; when TG levels exceeded 4.5 mmol/L, LDL-C values were considered missing [37]. AIP was calculated as (log[TG/HDL-C]). All blood sampling was performed after at least a 12-hour fast. *Apolipoprotein B* gene analysis (detection of the p.Arg3527Gln mutation) was performed in all subjects, followed by *LDL-receptor* gene analysis in patients without mutations in the *apolipoprotein B* gene, using the same stepwise approach described earlier [38].

### *Statistical analysis*

Standard descriptive statistics were used for the description of the dataset: the absolute and relative frequencies for categorical variables, arithmetic means for normally distributed variables and geometric means for log-normally distributed variables, accompanied by a confidence interval for continuous data. (The confidence interval has the same interpretation for the arithmetic and geometric means; in the case of the geometric mean, the confidence interval is asymmetric.) Prior to analysis, the assumption of normality was tested on continuous variables via histograms and a Kolmogorov–Smirnov test. The data were log-transformed in the case of log-normal distribution. The transformed data were used in subsequent calculations.

Differences in the frequencies of the categorical data were tested using Fisher's exact test. To test the differences between patients with different diagnoses in continuous parameters, an unpaired *t*-test was adopted. A separate analysis was performed for the original data, after adjustment for age and the effect of diabetes mellitus. The purpose of the adjustment was to equalize influence of age and diabetes on evaluated endpoints and to avoid artificially significant results caused by age and diabetes differences among groups of patients. Linear regression, with age and diabetes mellitus status as the explanatory variables, was used for adjusting. Additional analyses based on age and sex matched groups are provided for unbiased estimates of plasma lipids and atherogenic index of plasma in CVD + and CVD − group. The matching of groups was computed using propensity score matching when for each CVD + patient age and sex matching CVD − patients were selected with 1-to-2 matching. Analysis was performed with an IBM computer running SPSS Statistics 19.0.0 software (IBM Corporation, 2010) and R software with Nonrandom package (Susanne Stampf, 2011, Nonrandom: Stratification and matching by the propensity score).

## Results

A total of 555 FH patients (323 with mutations in the *LDLR* gene and 232 in the *APOB* gene) from our MedPed database were eligible for the present analysis. The basic characteristics of these populations are shown in Table 1. The LDLR and APOB groups did not differ in their age, sex, BMI, systolic and diastolic BP, cigarette smoking or presence of DM. There were higher plasma TC, LDL-C, Tg and lower HDL-C levels in LDLR group in comparison with APOB group (Table 1).

Fifty-three (42 with mutations in the *LDLR* gene and 11 in the *APOB* gene) of 555 FH patients had a history of CVD before entry into the database (CVD + group): 49 had coronary heart disease, 3 had stroke or transient ischemic attack and 1 had peripheral artery disease, while 502 FH patients had no history of CVD (CVD − group). As expected, FH patients in the CVD + group were significantly older in comparison with those in the CVD − group: 54.5 (51.2; 57.8) years vs. 39.6 (38.4; 40.8) years; $P<0.001$. The two groups did not differ significantly in their sex distribution or smoking habits. As there were more diabetics in the CVD + group compared to the CVD − group (4 [7.55%] vs. 7 [1.39%], $P=0.015$), BMI, BP, plasma lipids and AIP were adjusted for age and DM effects. Age and DM-adjusted BMI and systolic and diastolic BP did not differ significantly between the CVD + and CVD − groups (Table 2).

Nonadjusted, age-adjusted and age-DM-adjusted plasma lipids (TC, HDL-C, LDL-C and TG) and AIP in the CVD + and CVD − groups are shown in Table 3. Nonadjusted values of TC, LDL-C, TG and AIP were significantly higher in the CVD + group; the differences in HDL-C were much smaller but significant, favoring higher HDL-C in the CVD − group ($P=0.041$). Age-DM-adjusted TG and HDL-C levels were within the physiological ranges in both groups, but TG continued to be significantly higher ($P<0.001$) and HDL-C significantly lower ($P=0.017$) in the CVD + group. Conversely, age-DM-adjusted TC and LDL-C did not differ between the CVD + and CVD − groups.