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Original article

Size, density and cholesterol load of HDL predict microangiopathy, coronary artery disease and β-cell function in men with T2DM

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ABSTRACT

The role of high-density lipoprotein cholesterol (HDL-C) as modifiable risk factor for cardiovascular (CV) disease is increasingly debated, notwithstanding the finding that small-dense and dysfunctional HDL are associated with the metabolic syndrome and T2DM. In order to better clarify the epidemiological risk related to HDL of different size/density, without resorting to direct measures, it would seem appropriate to adjust HDL-C to the level of its main apolipoprotein (apoA-I), thereby providing an [HDL-C/apoA-I] ratio. The latter allows not only to estimate an average size for HDLs, but also to derive indices on particle number, cholesterol load, and density. So far, the potential usefulness of this ratio in diabetes is barely addressed. To this end, we sorted 488 male patients with T2DM according to [HDL-C/apoA-I] quartiles (Q), to determine how the ratio relates to cardiometabolic risk, β-cell function, glycaemic control, and micro- and macrovascular complications. Five lipid parameters were derived from the combined determination of HDL-C and apoA-I, namely HDL size; particle number; cholesterol load/particle; apoA-I/particle; and particle density. An unfavorable cardiometabolic profile characterized patients from QI and QII, in which HDLs were pro-atherogenic, denser and apoA-I-depleted. By contrast, QIII patients had an [HDL-C/apoA-I] ratio close to that of non-diabetic controls. QIV patients had better than average HDL size and composition, and in those patients whose [HDL-C/apoA-I] ratio was above normal, a more favorable phenotype was observed regarding lifestyle, anthropometry, metabolic comorbidities, insulin sensitivity, MetS score/severity, glycaemic control, and target-organ damage pregalence in small or large vessels. In conclusion, [HDL-C/apoA-I] and the resulting indices of HDL composition and functionality predict macrovascular risk and β-cell function decline, as well as overall microangiopathic risk, suggesting that this ratio could serve both in cardiometabolic assessment and as biomarker of vascular complications.

1. Introduction

There is an ongoing controversy about the role of high-density lipoprotein cholesterol (HDL-C) as modifiable risk factor for macrovascular disease. This debate, revived by the disappointing results of randomized clinical trials designed to increase HDL-C levels, has led to renewed interest towards particles' functionality. Indeed, the simple determination of HDL-C only represents an averaged and static measurement of the cholesterol load in different HDL subclasses. Further, the determination of HDL-C gives no indication as to the intensity of reverse cholesterol transport (RCT), or as to the functionality of HDL particles [1–7]. In the metabolic syndrome (MetS) and/or type 2 diabetes mellitus (T2DM), small-dense and/or dysfunctional HDL are prevalent, leading to less efficient RCT. These particulate anomalies are operating in the wider context of atherogenic dyslipidemia (AD), defined by the concurrence of low HDL-C and hypertriglyceridemia [8,9].

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Along with the approach which consists, for low-density lipoproteins (LDL), to measure the major particle's apolipoprotein ($apoB_{100}$) to better clarify the epidemiological risk related to LDL of different size/density, it would seem relevant to also adjust HDL-C level to HDL's major apolipoprotein, ie apolipoprotein A-I (apoA-I), as an [HDL-C/apoA-I] ratio, bearing in mind that unlike the single molecular ratio of $apoB_{100}$ vis-à-vis LDL, discoidal or spherical mature HDLs carry two or three apoA-I per particle [1,3–5].

Devising a [HDL-C/apoA-I] ratio allows not only to estimate an average size for HDLs, but also to derive complementary indices on particle number, cholesterol load, and density. This distinguishes patients in whom HDLs are less atheroprotective-proatherogenic due to over-representation of small-dense apoA-I-poor particles subclasses, namely HDL_{3a} [*very-small HDL* (HDL-VS)]; HDL_{3b} [*small HDL* (HDL-S)]; and HDL_{3c} [*medium HDL* (HDL-M)] without resorting to non-routine and costly quantification of HDL subclasses/functionality. The use of [HDL-C/apoA-I] ratio being recent enough, despite its obvious simplicity, its clinical use in humans is still in its infancy [10–16]. While T2DM represents a choice group, we are aware of only two studies having used [HDL-C/apoA-I] in such a high-risk population. Thus, [HDL-C/apoA-I] was associated with inflammatory biomarkers in patients with stable coronary artery disease (CAD) [11], while it was related to glycemic progression in the FIELD study, suggesting a link with residual β-cell function [12].

The aim of this work was as follows: (*i*) to phenotype T2DM Caucasians according to quartiles (Qs) of [HDL-C/apoA-I]; (*ii*) to determine how this ratio relates to cardiometabolic risk and glucose homeostasis; and (*iii*) to assess its association with prevalent cardiovascular complications, both micro- and macro-angiopathies. This work focuses on male subjects, as it would be inaccurate to perform a distribution analysis in a mixed gender cohort, on the grounds that HDL-C and apoA-I are sexually dimorphic, and to varying degrees, with average levels in men being decreased by 20% and 13% *vs.* women, respectively.

2. Patients and methods

The study was cross-sectional and included 488 Caucasians males with T2DM. Exclusion criteria included patients treated with medications that could substantially change insulin sensitivity (IS) or β-cell function, including systemic or topical corticosteroids, antiretroviral drugs, immune-modulatory drugs, and anti-psychotics. Were also excluded patients with chronic inflammatory diseases, cancer or major organ failure (respiratory, heart, and liver).

The following sociodemographic and clinical variables were recorded: age; highest educational attainment (as proxy for socio-economic status) based on four categories: (i) secondary school with leaving certificate (no graduation); (ii) school leaving certificate (with graduation); (iii) further education, but no degree; and (iv) university degree or similar, with highest educational attainment dichotomized as lower [(i)+(ii)] *vs.* higher [(iii)+(iv)]; diabetes duration, family history early-onset CHD (EOCHD) and/or diabetes mellitus (DM); smoking; ethanol intake; recreational physical activity; and leisure-time (LT) spent watching screens (television; personal computer or other visual numeric media) as surrogate for LT sedentarity. Non-alcoholic steatosis was considered in the presence of ultrasonic hyper-reflectivity in the absence of etiological factors associated with fatty liver, including excess ethanol intake. Obstructive sleep apnea/hypopnea syndrome (OSAS) prevalence was evaluated according to previously detailed criteria [17]. Medication use was recorded regarding glucose-lowering drugs (metformin; sulfonylureas/glinides; glitazones; dipeptidyl peptidase-4 inhibitors (DPP4-I); sodium-glucose transporter-2 inhibitors (SGLT2-I); glucagon-like peptide-1 receptor agonists (GLP-1-RA); insulin); and cardiovascular (CV) drugs (blood-pressure (BP)-lowering agents; aspirin (as antiplatelet agent); lipid-modifying drugs (LMD): statins; fibrates and/or ezetimibe).

The presence of a MetS was defined as a score ≥3/5 for the five following items: (*i*) impaired fasting glucose or glucose intolerance or diabetes mellitus; (*ii*) hypertension; (*iii*) enlarged waist; (*iv*) elevated fasting TG; and (*v*) decreased HDL-C, according to the *IDF-NHLBI-AHA-WHF-IAS-IASO* definition [18]. Each subject underwent a non-invasive combined assessment of β-cell function (B) and IS using the Homeostasis Model Assessment (HOMA-2, computer-based version: http://www.dtu.ox.ac.uk), from triplicates means of fasting glucose and specific insulin levels obtained after an overnight fast and discontinuation of all glucose-lowering or glucose-sensitizing therapies for 24 h (48 h in case of insulin glargine and long-acting sulfonylureas and 7–28 days in case of glucagon-like peptide 1 receptor agonists with long duration of action). For patients treated with glitazones, HOMA was performed prior to introduction of the long-acting insulin-sensitizer [19,20]. Values of insulin secretion ([B]; normal value 100%) were plotted as a function of insulin sensitivity ([S]; normal value 100%), defining a *hyperbolic product* area [B × S] (unit: $\%^2$; normal: 100%, corresponding to $10^4\%^2$), representing the true, underlying β-cell function. The secular loss of hyperbolic product ([BxS] loss rate ($\%\,year^{-1}$)) was obtained by dividing (100%-[BxS]) by each participant's age at the time of HOMA [21,22].

Glucose-related polyneuropathy, retinopathy, and nephropathy were defined using ICD-9-CM diagnoses and procedure codes, with diabetic nephropathy also identified using estimated glomerular filtration rate (eGFR) values $<60\,mL/min/1.73\,m^2$, defined as reduced kidney function. Since the latter may not *de facto* be attributable to diabetes, any eGFR-identified overt nephropathy was considered to represent diabetic nephropathy in the absence of a confirmed diagnosis of non-specific, non-diabetic nephropathy. The presence of a peripheral neuropathy was based on clinical examination (knee and ankle reflexes; Semmes-Weinstein monofilament test) and/or electromyography. Eye visual examinations by an experienced ophthalmologist and/or fluorescein angiography were performed to diagnose retinopathy.

Coronary artery disease (CAD) was defined from medical history (myocardial infarction, angioplasty, stenting, revascularization surgery and/or significant coronary stenosis confirmed by angiography); systematic review of procedures; and/or following screening with exercise testing, echocardiography, magnetic resonance imaging, or other subclinical disease imaging techniques. Cerebrovascular disease was considered in case of transient ischaemic attack or stroke, the latter defined by any neurological deficit ≥1 month, without distinction between ischemic, embolic and haemorrhagic events. Peripheral artery disease (PAD) was diagnosed from medical history of lower-limb(s) claudication; clinical or imaging evidence for ischemic diabetic foot; history of angioplasty, stenting, revascularization surgery; and/or lower-limb artery stenosis at Doppler ultrasonography or angiography.

The following laboratory variables were measured: HbA_{1c}; fasting glucose and insulinemia (after 48 h of glucose-lowering drug(s) discontinuation in T2DM); $_{hs}$CRP; cystatin C; liver enzymes (*aspartate aminotransferase* [AST], *alanine aminotransferase* [ALT]), *γ-glutamyl transferase* [γGT]; thyroid stimulating hormone (TSH); and *sex hormone-binding globulin* (SHBG). All lipids and lipoproteins values were obtained from fasting samples: total cholesterol (C), HDL-C, triglycerides (TG); LDL-C (computed using Friedewald's formula), non-HDL-C (by subtracting HDL-C from total C), and lipoprotein(*a*). ApoA-I and $apoB_{100}$ (apoB) were determined with immunonephelometry on BNII Analyzer® (Siemens Healthcare Products GmbH, Marburg, Germany). The within-subject coefficients of variation were: 6.9% (apoB), 6.5%

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