Biochimica et Biophysica Acta 1801 (2010) 89–94

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Atorvastatin treatment lowers fasting remnant-like particle cholesterol and LDL subfraction cholesterol without affecting LDL size in type 2 diabetes mellitus: Relevance for non-HDL cholesterol and apolipoprotein B guideline targets

Paul J.W.H. Kappelle [a], Geesje M. Dallinga-Thie [b], Robin P.F. Dullaart [a,*] and for the Diabetes Atorvastatin Lipid Intervention (DALI) study group [1]

[a] Department of Endocrinology, University Medical Center Groningen, P.O. Box 30001 9700 RB Groningen, The Netherlands
[b] Laboratory of Experimental Vascular Medicine, Academic Medical Center Amsterdam, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 8 May 2009
Received in revised form 11 September 2009
Accepted 24 September 2009
Available online 2 October 2009

Keywords:
Apolipoprotein B
atorvastatin
Diabetes mellitus
LDL-cholesterol
LDL subfractions
Non-HDL-cholesterol
Remnant-like particle cholesterol
Small dense LDL
Triglycerides

ABSTRACT

The extent to which atorvastatin treatment affects LDL size, LDL subfraction levels and remnant-like particle cholesterol (RLP-C) was determined in type 2 diabetes. We also compared LDL size and RLP-C in relation to guideline cut-off values for LDL cholesterol, non-HDL cholesterol and apolipoprotein (apo) B. Changes in LDL size and RLP-C were determined in fasting plasma from type 2 diabetic patients after 30 weeks administration of atorvastatin (10 mg daily, $n=65$; 80 mg daily, $n=62$) or placebo ($n=58$). LDL subfraction cholesterol was measured in 74 participants. Atorvastatin lowered LDL cholesterol, non-HDL cholesterol, triglycerides, apo B and RLP-C ($P<0.001$ for all at each dose) and LDL mean peak particle diameter remained unchanged. Atorvastatin treatment decreased cholesterol concentrations in all LDL subfractions ($P<0.001$ for each dose). RLP-C at follow-up was lower in those patients achieving the non-HDL cholesterol or the apo B guideline targets ($P<0.01$), but the LDL cholesterol cut-off value failed to discriminate. In conclusion, atorvastatin lowers fasting RLP-C and LDL subfraction cholesterol in diabetes. The proposed guideline cut-off levels for non-HDL cholesterol and apo B may be superior to the LDL cholesterol target in discriminating between higher and lower RLP-C levels.

1. Introduction

It is generally appreciated that the increased risk of cardiovascular disease in Type 2 diabetes mellitus is in part attributable to abnormalities in the quantity as well as in the quality of plasma lipoproteins [1–4]. Besides elevated triglycerides and high density lipoprotein cholesterol (HDL-C), diabetic dyslipidemia is also characterized by a predominance of small dense low density lipoprotein (LDL) particles (pattern B as opposed to pattern A phenotype) [5–7]. Small dense LDL particles are associated with insulin resistance and high triglycerides, and have been linked to increased atherosclerosis susceptibility [6,7]. However, it is uncertain whether LDL size predicts incident cardiovascular disease when plasma levels of LDL cholesterol (LDL-C), HDL-C and triglycerides are taken into account [8]. Plasma also contains remnant particles derived from triglyceride-rich, apolipoprotein (apo) B-containing lipoproteins (very low density lipoproteins (VLDL) and chylomicrons) which are considered to be atherogenic as well [9]. The remnant-like particle (RLP) cholesterol concentration, measured using an immuno-affinity technique that detects VLDL and chylomicron remnants, has been shown to be elevated in type 2 diabetes [10]. Importantly, the RLP cholesterol (RLP-C) concentration may predict recurrent cardiovascular disease even after controlling for hypercholesterolemia, hypertriglyceridemia and low HDL-C [11].

A recent meta-analysis has convincingly demonstrated that statin treatment lowers vascular mortality in diabetic patients [12]. Current guidelines recommend early use of statin therapy for primary cardiovascular risk prevention in this patient category [13], and aggressive LDL-C lowering results in additional cardiovascular benefit [14]. Since there remains considerable residual risk during treatment, it is clinically relevant to document the extent to which LDL particle size, LDL subfraction cholesterol levels and RLP-C concentration is affected by statin treatment in diabetic subjects. Equivocal effects of statin therapy have been reported on LDL size and subfraction distribution in subjects with diabetes [15–21], whereas limited data are available with respect to the effect of this treatment on RLP-C [17,19]. Moreover, although the triglyceride lowering by statin treatment may be dose-dependent in hypertriglyceridemic subjects [22], effects on LDL quality and RLP-C have not been compared between usual and high dose statin administration in diabetes mellitus.

* Corresponding author. Tel.: +31503613731; fax: +31 503619392.
E-mail address: r.p.f.dullaart@int.umcg.nl (R.P.F. Dullaart).
[1] The members of the DALI study group are listed in the appendix.

1388-1981/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2009.09.021

Table 1
Clinical characteristics and low density lipoprotein cholesterol (LDL-C), non-high density lipoprotein cholesterol (non-HDL-C), apolipoprotein (apo) B and triglycerides at baseline and after placebo or atorvastatin treatment (10 mg or 80 mg daily) in type 2 diabetic patients.

	Placebo ($n=58$)		Atorvastatin 10 mg ($n=65$)		Atorvastatin 80 mg ($n=62$)		§P-value
Age (years)	55 ± 7		60 ± 7		60 ± 8		0.50
Fasting glucose (mmol/l)	10.0 ± 2.4		10.2 ± 2.5		10.9 ± 3.1		0.83
HbA1c (%)	8.3 ± 1.1		10.2 ± 2.5		8.4 ± 1.1		0.72
Sex (men/women)	29/29		41/24		33/29		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	
LDL-C (mmol/l)	3.65 ± 0.88	3.59 ± 0.85	3.65 ± 0.91	2.15 ± 0.58*	3.72 ± 0.95	1.73 ± 0.99*	<0.001
Non-HDL-C (mmol/l)	4.95 ± 0.74	4.91 ± 0.85	4.86 ± 0.90	2.98 ± 0.69*	5.02 ± 0.93	2.55 ± 1.04*	<0.001
Apo B (g/l)	1.26 ± 0.19	1.24 ± 0.20	1.21 ± 0.20	0.83 ± 0.16*	1.25 ± 0.24	0.74 ± 0.25*	<0.001
Triglycerides (mmol/l)	2.57 (2.06–3.33)	2.31 (1.86–3.30)	2.44 (1.90–3.40)	1.63 (1.27–2.13)*	2.61 (2.06–3.65)	1.54 (1.05–2.01)*	<0.001

Data in mean ± SD or in median (interquartile range). *P<0.001 compared to baseline; §P-value for between group differences (in change) by ANOVA.

The present study was carried out to determine the effect of usual and high dose atorvastatin treatment on fasting LDL size and LDL subfraction cholesterol levels, as well as on RLP-C in type 2 diabetic patients participating in the DALI (Diabetes Atorvastatin Lipid Intervention) trial. It has been advocated recently to use non-HDL cholesterol (non-HDL-C) and apo B as primary treatment targets in addition to LDL-C with plasma triglycerides being a secondary goal [13,23]. We, therefore, also compared LDL size and RLP-C in relation to the guideline cut-off values for LDL-C, non-HDL-C, apo B and triglycerides.

2. Patients and methods

2.1. Participants

The DALI study is a prospective, randomized multicenter study, which is aimed to demonstrate the effect of low (10 mg daily) and high dose (80 mg daily) atorvastatin treatment on plasma lipids and lipoproteins in patients with type 2 diabetes mellitus [24]. This trial has been carried out in 3 centers in The Netherlands (University Medical Centers of Leiden, Rotterdam and Utrecht). Approval of the medical ethics committees of each participating center had been obtained, and all participants had provided written informed consent. Full details of the design of the study have been published previously [24]. In short, men and women aged 45–75 years, with a known duration of type 2 diabetes of at least 1 year, hypertriglyceridemia (fasting triglycerides between 1.5 and 6.0 mmol/l) and a plasma total cholesterol between 4.0 and 8.0 mmol/l were included. An HbA_{1c} above 10%, a history of cardiovascular disease, hepatic disease, renal insufficiency, as well as malignancy, systemic inflammatory disease, gastrointestinal disease and excessive alcohol use were the most important exclusion criteria. If applicable, lipid-lowering therapy was withdrawn 8 weeks before start of the run-in phase. Atorvastatin and matching placebo were given in the morning. In patients randomized to atorvastatin 80 mg daily, this statin was started at a dose of 40 mg daily and then increased to 80 mg daily after 4 weeks. The present study included 58 patients randomized to placebo, 65 to atorvastatin 10 mg daily and 62 randomized to atorvastatin 80 mg daily who completed follow-up and from whom RLP measurements were available. LDL subfraction isolation and cholesterol measurements were carried out in 74 patients who were randomly selected from the cohort. Participants were studied after an overnight fast of at least 12 h at baseline and after 30 weeks of follow-up. Of the study subjects 2% were treated with diet alone, 44% with oral glucose lowering drugs, 28% with insulin and 26% with combination therapy. Treatment for diabetes was equally distributed among the 3 randomized groups.

2.2. Laboratory measurements

Venous blood was collected in EDTA containing tubes. Plasma was obtained by centrifugation at 3000 rpm for 15 min at 4 °C and the samples were stored at −80 °C until analysis. Total cholesterol and triglycerides were measured by enzymatic colorimetric methods as described [24]. HDL-cholesterol (HDL-C) was measured by a direct enzymatic assay based on a polyethyleneglycol-modified method (Boehringer Mannheim; Hitachi 911 analyzer). LDL-C was calculated by the Friedewald formula [25], and non-HDL-C was calculated as the difference between total cholesterol and HDL-C. Apo B was determined with an immunoturbidimetric assay (Tina-quant apo B, cat. no. 1551779, Boehringer Mannheim; Hitachi 917 analyzer) with calibration according to International Federation of Clinical Chemistry standards [26]. LDL particle size was measured by polyacrylamide gradient gel electrophoresis [27]. Standardization was achieved by inclusion of LDL samples with known size donated by Ronald M. Krauss. Based on their peak size, particles were divided into two classes: particles >25.5 nm (pattern A) reflect the presence of predominantly large, buoyant LDL particles, and particles <25.5 nm (pattern B) with a predominance of small LDL particles.

LDL subfractions were isolated by density gradient ultracentrifugation using a six-step, discontinuous salt gradient. Twenty 500 - µl aliquots were collected after centrifugation in a Beckman SW40

Table 2
Low density lipoprotein (LDL) size pattern, LDL particle size and remnant like particle cholesterol (RLP-C) concentration at baseline and after placebo or atorvastatin treatment (10 mg or 80 mg daily) in type 2 diabetic patients.

	Placebo ($n=58$)		Atorvastatin 10 mg ($n=65$)		Atorvastatin 80 mg ($n=62$)		§P-value
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	
LDL particle size pattern (A/B)	41/17*	41/17**	42/23*	50/15**	38/24*	42/20**	
LDL peak particle diameter (nm)	25.90 ± 0.72	25.94 ± 0.62	25.87 ± 0.75	26.01 ± 0.71	25.76 ± 0.75****	25.92 ± 0.73	0.63
RLP-C (mmol/l)	0.45 (0.30-0.64)	0.37 (0.28-0.49)	0.43 (0.33-0.70)	0.25 (0.17-0.37)***	0.47 (0.32-0.64)	0.21 (0.17-0.33)***	< 0.001

Data in mean ± SD or in median (interquartile range). LDL size pattern distribution by Chi-square analysis: *baseline: $P=0.68$; **follow-up: $P=0.63$. ***P<0.001 compared to baseline. ****P<0.02 compared to baseline. §P-value for between group difference in change by ANOVA.