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Dyslipidemia, but not hyperglycemia and insulin resistance, is associated with marked alterations in the HDL lipidome in type 2 diabetic subjects in the DIWA cohort: Impact on small HDL particles

Marcus Ståhlman [a], Björn Fagerberg [a], Martin Adiels [a], Kim Ekroos [b], John M. Chapman [c], Anatol Kontush [c,1], Jan Borén [a,*,1]

[a] Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, Göteborg University, Göteborg, Sweden
[b] Zora Bioscience, Espoo, Finland
[c] Dyslipidemia, Inflammation and Atherosclerosis Research Unit (UMR 939), INSERM, and University of Pierre and Marie Curie, Paris 6, Paris, F-75013 France

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ABSTRACT

In this study we have used mass spectrometry in order to characterize the HDL lipidome in three groups of women from the DIWA cohort; one control group, plus two groups with type 2 diabetes with insulin resistance; one dyslipidemic and one normolipidemic. The aim was to investigate whether dyslipidemia is required in addition to insulin resistance for the occurrence of an altered HDL lipidome, which in turn might impact HDL functionality. The dyslipidemic type 2 diabetic subjects were distinguished by obesity, hypertriglyceridemia with elevated apoC3, low HDL-cholesterol and chronic low grade inflammation. In a stepwise multivariate linear regression analysis, including biomarkers of dyslipidemia and insulin resistance as independent variables, only dyslipidemia showed a significant correlation with HDL lipid classes. Small HDL-particles predominated in dyslipidemic subjects in contrast to the normolipidemic diabetic and control groups, and were enriched in lysophosphatidylcholine (+13%), a product of proinflammatory phospholipases, and equally in two core lipids, palmitate-rich triacylglycerols and diacylglycerols (+77 %), thereby reflecting elevated CETP activity. Dyslipidemic small HDL particles were further distinguished not only as the primary carrier of ceramides, which promote inflammation and insulin resistance, but also by a subnormal plasmalogen/apoAI ratio, consistent with elevated oxidative stress typical of type 2 diabetes. From these data we conclude that in type 2 diabetes, dyslipidemia predominates relative to hyperglycemia for the occurrence of an altered HDL lipidome. Furthermore, dyslipidemia alters the cargo of bioactive lipids, with implications for HDL function.

1. Introduction

Perturbed intravascular lipid and lipoprotein metabolism is a common feature of type 2 diabetes, leading to a dyslipidemia involving elevated plasma concentrations of triglycerides and apolipoprotein (apo) B-containing lipoproteins (VLDL, VLDL remnants and LDL) and subnormal levels of HDL, a lipid phenotype associated with accelerated atherosclerosis and high cardiovascular risk. While apoB-containing lipoproteins are atherogenic, HDL exerts athero- and vasculo-protective effects [1,2]. HDL-mediated atheroprotection has been principally attributed to the cellular cholesterol efflux capacity of these particles [3–5]. Recently however, the antioxidative, antiinflammatory and vasodilatory activities of HDL have equally been shown to exert atheroprotective effects [6]. Among the lipid and protein components of HDL particles, the major apolipoprotein, apoA-I, plays a central role in such activities [5,7].

Significantly, the athero- and vasculoprotective properties of HDL are frequently compromised in insulin-resistant, type 2 diabetic subjects [8–13]. Indeed, defective HDL function in type 2 diabetic subjects can result from alterations in both its protein and lipid components (proteome and lipidome respectively) [3]. In type 2 diabetes, the HDL particle content of apoA-I is frequently reduced [14]. Furthermore, elevated plasma activity of cholesteryl ester transfer rotein (CETP) in type 2 diabetic subjects leads to HDL-enrichment with triacylglycerol (TG) and depletion of cholesteryl ester (CE) [15].

Abbreviations: Apo, Apolipoprotein; CE, Cholesterol ester; TG, Triacylglycerol; FC, Free cholesterol; DG, Diacylglycerol; SM, Sphingomyelin; CER, Ceramide; LPC, Lysophosphatidylcholine; PC, Phosphatidylcholine; PC-O, Ether-linked phosphatidylcholine; CETP, Cholesteryl ester transfer protein; QUICKI, Quantitative insulin sensitivity check index; CID, Collision-induced dissociation

* Corresponding author at: Wallenberg Laboratory, Sahlgrenska University Hospital, S-413 45 Gothenburg, Sweden. Tel.: +46 31 3422949; fax: +46 31 823762.
E-mail address: Jan.Boren@wlab.gu.se (J. Borén).
[1] These authors contributed equally.

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Such alterations in the HDL lipidome may impact the conformation of apoA-I secondary to CETP-mediated modification, thereby contributing to HDL dysfunction [9,12,16]. Indeed, the deficient antioxidative and vasodilatory activities of diabetic HDL correlate with their enrichment in TG and depletion of CE [9,12].

Until now, only superficial features of the lipidome of HDL in diabetic subjects have been assessed, i.e. at the level of the major esterified lipid classes (CE, TG phospholipids), thereby failing to resolve component molecular lipid species, and more specifically, those possessing biological activities. Indeed, given the potent biological activities of plasma HDL particles, the quantity and quality of specific HDL lipid species may facilitate identification of perturbed biological pathways relevant to cardiovascular disease in type 2 diabetes. Quantitative and qualitative profiling of such HDL lipid species will therefore advance our understanding both of the lipidome and of the pathophysiology of accelerated atherosclerosis in dyslipidemic type 2 diabetic subjects, and equally will provide insight into structure-function relationships in HDL in type 2 diabetes. In this way, novel lipid biomarkers of perturbed metabolism and premature atherosclerosis can be identified in type 2 diabetes by lipidomics technologies.

Type 2 diabetes involves hyperglycemia and insulin resistance and is typically accompanied by atherogenic dyslipidemia featuring elevated levels of TG-rich lipoproteins and small, dense LDL, with subnormal HDL levels. The degree to which hyperglycemia or dyslipidemia, or both, impact alteration in the lipidome of HDL with associated dysfunction is indeterminate. We hypothesized that perturbation of the HDL lipidome with altered biological activity may result from the association of dyslipidemia with insulin resistance, rather than insulin resistance alone. To evaluate this question, we identified three subgroups of post-menopausal females within the DIWA cohort (Diabetes and Impaired glucose tolerance in Women and Atherosclerosis) [17]; subjects in each group were extensively phenotyped with respect to family history of cardiometabolic disease, and anthropometric, clinical and biological parameters. These groups consisted of (i) control, normolipidemic subjects, (ii) normolipidemic subjects but with type 2 diabetes as defined by the World Health Organisation (11), and a third group characterized by the same inclusion criteria as group (ii), but in addition, this last group displayed diabetic dyslipidemia (\ 1). A lipidomics approach revealed that dyslipidemia, rather than hyperglycemia and insulin resistance, is the major factor in inducing perturbation in functionally relevant components of the HDL lipidome in type 2 diabetes.

2. Materials and methods

2.1. Materials and chemicals

Cholesteryl oleate, triolein and cholesterol were acquired from Larodan (Malmö, Sweden) and used as external standards for HPLC analysis. 1,2-diheptadecanoyl-*sn*-glycerol from Larodan (Malmö, Sweden) and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine, 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, N-heptadecanoyl-D-*erythro*-sphingosine and N-heptadecanoyl-D-*erythro*-sphingosylphosphorylcholine (Avanti Polar Lipids Inc., Alabaster, AL) were used as internal standards for MS analysis. External ceramide standards with C16:0, C18:0, C24:0 and C24:1 fatty acid moieties were acquired from Larodan (Malmö, Sweden). Lanosterol was purchased from MP Biomedicals (Solon, OH) and used as an internal standard for the HPLC analysis.

2.2. Study participants

The subjects included in this study were all 64-year old post-menopausal women, originally part of the DIWA study (Diabetes and Impaired glucose tolerance in Women and Atherosclerosis) [17]. From this cohort, we defined and selected three groups of subjects ($n = 20$ in each): (1) Control subjects (HOMA < 1.35) with normal blood lipids (TGs < 1.7 mM and HDL-C > 1.29 mM), (2) subjects with normal blood lipids (TGs < 1.7 mM and HDL-C > 1.29 mM) but with type 2 diabetes as defined by the World Health Organization [18], insulin resistance (HOMA > 1.35) and glutamic acid decarboxylase antibodies < 4.6 units ml^{-1}; (3) The third group fulfilled both the same inclusion criteria as group 2 and criteria for diabetic dyslipidemia (TGs > 1.7 mM and HDL-C < 1.29 mM). Blood samples were withdrawn after overnight fasting. No subject was treated with lipid-lowering or anti-diabetic drugs. From the female subjects who matched these criteria, 20 were randomly selected to constitute each subgroup (Table 1).

2.3. Lipoprotein isolation

HDL (d = 1.063–1.210 g/ml) was isolated from 500 μl EDTA plasma by sequential ultracentrifugation using heavy water and sucrose according to previous work [19]. The amount of isolated lipoproteins was evaluated by analyzing apoA-I concentrations using immunoturbidimetric methods on a Konelab 20 autoanalyser (Thermo Electron Corporation, Vantaa, Finland).

2.4. Lipid extraction

Lipids were extracted from HDL according to a modified Folch procedure [20]. Briefly, 75 μl of the recovered HDL was extracted with 500 μl methanol and 1000 μl chloroform containing internal standards. After 10 minutes of vortex mixing, 300 μl of 20 mM acetic acid was added. After 10 minutes of vortex mixing, the organic phase was transferred to a glass vial and the remaining water phase washed with 500 μl of chloroform. The organic phases were pooled and evaporated under a stream of nitrogen. The extract was reconstituted in

Table 1
Clinical and biological characteristics of post-menopausal subjects in the control group, the normolipidemic, hyperglycemic type 2 diabetic group and the dyslipidemic, hyperglycemic type 2 diabetic group from the DIWA cohort.

	CTRL ($n = 20$)	T2D ($n = 20$)	T2D + DL ($n = 20$)
BMI (kg/m^2)	24 ± 3.3	28 ± 2.7*	32 ± 4.5*†
Waist (cm)	85 ± 6.0	96 ± 8.7*	107 ± 8.5*†
Triacylglycerols (mmol/l)	1.0 ± 0.2	1.2 ± 0.3	2.8 ± 1.2*†
HDL-C (mmol/l)	1.9 ± 0.2	1.7 ± 0.3	1.1 ± 0.2*†
LDL-C (mmol/l)	3.3 ± 0.7	3.1 ± 1.1	4.0 ± 1.0†
HOMA	0.85 ± 0.3	3.8 ± 1.6*	5.4 ± 3.7*
QUICKI	0.40 ± 0.03	0.33 ± 0.02*	0.31 ± 0.02*
HbA_{1c} (%)	5.5 ± 0.3	6.2 ± 0.7*	7.0 ± 1.6*
HbA_{1c} (mmol/mol)	37 ± 2.9	45 ± 7.5*	53 ± 18*
ApoC3 (mg/dl)	13 ± 2.5	12 ± 2.6	18 ± 5.5*†
C-reactive protein (nM)	14 ± 12	23 ± 36	36 ± 43
Glucose (mM)	4.7 ± 0.6	6.4 ± 1.1*	7.4 ± 2.3*
Insulin (pM)	28 ± 8.2	87 ± 35*	103 ± 48 *
ApoA-I (mg/dl)	165 ± 21	156 ± 16	137 ± 81*†
ApoB100 (mg/dl)	101 ± 16	102 ± 26	149 ± 32*†
Lp(a) (mg/dl)	22 ± 18	51 ± 58	42 ± 35
Serum creatinine (μmol/l)	85.7 ± 7.0	85.5 ± 10.1	88.9 ± 9.4
Urinary albumin (mg/l)	2.9 ± 2.3	5.5 ± 6.4	7.2 ± 5.2*
Urinary creatinine (nmol/l)	6.0 ± 2.2	6.0 ± 3.0	6.7 ± 3.2
ICAM (ng/ml)	241 ± 32	271 ± 69	342 ± 113*†
E-selectin (ng/ml)	28.8 ± 8.0	30.4 ± 16.2	47.1 ± 24.4*†
Systolic BP (mm Hg)	128 ± 21	145 ± 23*	144 ± 17*
Diastolic BP (mm Hg)	74 ± 11	81 ± 11*	82 ± 8*
Leptin (ng/ml)	0.18 ± 0.14	0.36 ± 0.20	0.40 ± 0.19*
Adiponectin (μg/ml)	1.9 ± 0.7	1.2 ± 0.6*	0.86 ± 0.55*†

T2D = normolipidemic type 2 diabetic subjects; T2D + DL = dyslipidemic type 2 diabetic subjects. Values are mean ± SD.
* $p < 0.05$ vs. CRTL.
† $p < 0.05$ vs. T2D.

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