



Contents lists available at ScienceDirect

Environment International

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

Full length article

Alterations in high-density lipoprotein proteome and function associated with persistent organic pollutants

Stefan A. Ljunggren^{a,*}, Ingela Helmfrid^a, Ulf Norinder^b, Mats Fredriksson^c, Gun Wingren^c, Helen Karlsson^a, Mats Lindahl^c

^a Occupational and Environmental Medicine Center, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

^b Swedish Toxicology Sciences Research Center, Södertälje, Sweden

^c Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

ARTICLE INFO

Article history:

Received 20 July 2016

Received in revised form 16 October 2016

Accepted 5 November 2016

Available online xxxx

Keywords:

Cardiovascular disease

Environmental

HDL

Lipoproteins

Persistent organic pollutants

ABSTRACT

There is a growing body of evidence that persistent organic pollutants (POPs) may increase the risk for cardiovascular disease (CVD), but the mechanisms remain unclear. High-density lipoprotein (HDL) acts protective against CVD by different processes, and we have earlier found that HDL from subjects with CVD contains higher levels of POPs than healthy controls. In the present study, we have expanded analyses on the same individuals living in a contaminated community and investigated the relationship between the HDL POP levels and protein composition/function.

HDL from 17 subjects was isolated by ultracentrifugation. HDL protein composition, using nanoliquid chromatography tandem mass spectrometry, and antioxidant activity were analyzed. The associations of 16 POPs, including polychlorinated biphenyls (PCBs) and organochlorine pesticides, with HDL proteins/functions were investigated by partial least square and multiple linear regression analysis.

Proteomic analyses identified 118 HDL proteins, of which ten were significantly ($p < 0.05$) and positively associated with the combined level of POPs or with highly chlorinated PCB congeners. Among these, cholesteryl ester transfer protein and phospholipid transfer protein, as well as the inflammatory marker serum amyloid A, were found. The serum paraoxonase/arylesterase 1 activity was inversely associated with POPs. Pathway analysis demonstrated that up-regulated proteins were associated with biological processes involving lipoprotein metabolism, while down-regulated proteins were associated with processes such as negative regulation of proteinases, acute phase response, platelet degranulation, and complement activation.

These results indicate an association between POP levels, especially highly chlorinated PCBs, and HDL protein alterations that may result in a less functional particle. Further studies are needed to determine causality and the importance of other environmental factors. Nevertheless, this study provides a first insight into a possible link between exposure to POPs and risk of CVD.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Environmental pollution is a widespread problem affecting people worldwide. The World Health Organization has recently estimated that 30% of the disability-adjusted life years lost due to cardiovascular diseases (CVD) is caused by environmental factors. In addition, CVD in the form of stroke and ischemic heart disease were the diseases with the strongest environmental contribution (Prüss-Üstün et al., 2016). Possible important environmental factors in this respect are the persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and dioxins. POPs are lipophilic man-made compounds that bio-accumulate and are stored in fat tissues. Several POPs are recognized as endocrine-disrupting chemicals and an increasing amount of data is supporting a role for POPs in the development of CVD (Lind and Lind, 2012). In addition, as recently

Abbreviations: Apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; DCFH, 2,7-dichlorofluorescein; DCF, 2,7-dichlorofluorescein; GO, gene ontology; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HDL, HDL oxidant index; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; MS, mass spectrometry; OCPs, organochlorine pesticides; PCB, polychlorinated biphenyl; PON1, serum paraoxonase/arylesterase 1; POP, persistent organic pollutant; PLS, partial least squares; PLTP, phospholipid transfer protein; p,p'-DDE, 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; SAA, serum amyloid A; sumPCB-HC, sum of highly chlorinated PCB congeners; sumPOP, sum of persistent organic pollutants; VIP, variable importance in projection; VLDL, very low-density lipoprotein.

* Corresponding author: Stefan Ljunggren, Occupational and Environmental Medicine, Heart Medical Centre, Linköping University Hospital, SE-581 85 Linköping, Sweden.

E-mail addresses: stefan.ljunggren@liu.se (S.A. Ljunggren), ingela.helmfrid@regionostergotland.se (I. Helmfrid), ulf.norinder@swetox.se (U. Norinder), mats.fredriksson@liu.se (M. Fredriksson), gun.wingren@liu.se (G. Wingren), helen.m.karlsson@liu.se (H. Karlsson), mats.lindahl@liu.se (M. Lindahl).

<http://dx.doi.org/10.1016/j.envint.2016.11.008>

0160-4120/© 2016 Elsevier Ltd. All rights reserved.

Please cite this article as: Ljunggren, S.A., et al., Alterations in high-density lipoprotein proteome and function associated with persistent organic pollutants, Environ Int (2016), <http://dx.doi.org/10.1016/j.envint.2016.11.008>

reviewed, PCBs are known to affect several signaling pathways leading to oxidative stress and inflammation with implications for CVD risk (Perkins et al., 2016). In a cross-sectional study of the NHANES cohort, an association between POPs and CVD were found in women (Ha et al., 2007). Cross-sectional studies of the PIVUS cohort have suggested an association between POPs and inflammatory markers (Kumar et al., 2014a) as well as increased oxidative stress (Kumar et al., 2014b). In the same population, POPs have been shown to be prospectively associated with lipid values such as low-density lipoprotein (LDL) cholesterol (LDL-C), a well-established risk factor for CVD (Penell et al., 2014).

An integral part of the lipid metabolism are lipoproteins that consist of proteins and phospholipids encapsulating a lipid-rich core of cholesteryl ester. They are classified according to their density mainly into high-density lipoprotein (HDL), LDL or very low-density lipoprotein (VLDL). HDL has an important function in the reverse cholesterol transport in which cholesterol from peripheral cells are transported to the liver for possible excretion into bile (Rosenson et al., 2012). HDL is regarded as protective against CVD due to the reverse cholesterol transport as well as many other atheroprotective functions such as anti-inflammatory and anti-thrombotic effects (Annema and von Eckardstein, 2013). In addition, HDL has been shown to reduce cytokine-induced inflammatory markers on endothelial cells (Cockerill et al., 1995) and inhibit the oxidation of LDL (Mackness et al., 1991, Navab et al., 2000a). HDL is also known to detoxify environmental pathogens and toxins (Karlsson et al., 2015). The particles are however heterogeneous and shifting in their composition. Therefore, they may lose protective functions or even become dysfunctional/pro-inflammatory under certain conditions such as acute coronary artery disease, most likely due to changes in their protein composition (Annema and von Eckardstein, 2013, Rosenson et al., 2016). HDL protein profiling has consequently become an important tool in CVD research and prominent proteins include apolipoprotein (apo) A-I (apoA-I), which is the main structural protein in HDL (Davidson et al., 2009) and serum paraoxonase/arylesterase 1 (PON1) that has protective properties against oxidative stress (Precourt et al., 2011). Both of these proteins have been shown to prevent the oxidation of LDL, a process in atherosclerosis, within human artery wall cells (Navab et al., 2000b).

We have previously studied a contaminated community in Sweden and the association between exposure to POPs and cancer (Helmfrid et al., 2012, Helmfrid et al., 2015). In a separate study of the same area we have shown that POPs in plasma are transported in the lipoprotein fractions of HDL and LDL/VLDL and that highly chlorinated PCB congeners (octa-, nona- and deca-chlorinated) have a higher propensity to be located in lipoproteins compared to less chlorinated congeners (Ljunggren et al., 2014). In the same study, we also found higher lipoprotein POP levels in subjects with CVD than healthy individuals. In the present study, we have therefore used a proteomic approach on a subsample of the same population to investigate associations between POPs and the HDL proteome that may provide mechanistic links between environmental exposure to POPs and an increased risk of CVD.

2. Methods and material

2.1. Sample

Samples from a previous study regarding health effects related to POPs and metals in a Swedish community were used (Helmfrid et al., 2012). Previously, the levels of POPs, including 14 PCB congeners and two OCPs; 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (p,p'-DDE) and trans-nonachlor, were measured and identified in the HDL fraction (Ljunggren et al., 2014). In this follow-up study, the HDL protein composition from seven healthy controls and ten individuals with CVD was investigated (Table 1). HDL-C was measured with a HDL quantitation kit (MAK045, Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions. The study was approved by the local ethics committee.

Table 1
Clinical characteristics and POP levels in participants.

	All (n = 17)	Healthy (n = 7)	CVD (n = 10)
Age	67 (41–87)	62 (41–79)	77 (57–87)
Gender (M/F)	9/8	3/4	6/4
BMI	27 (20–33)	30 (24–31)	26 (20–33)
Triglycerides (mmol/L)	1.8 (0.8–2.8)	2.2 (1–2.8)	1.8 (0.8–2.7)
Cholesterol (mmol/L)	5.8 (3.9–8.2)	5.9 (4.9–8.2)	5.5 (3.9–6.9)
HDL-C (mmol/L)	0.8 (0.5–1.6)	0.8 (0.5–1.6)	0.8 (0.5–1.4)
POP values (pg/mL isolated HDL)			
PCB#74	10.5 (9.6–22.4)	9.8 (9.8–9.8)	11.2 (9.6–22.4)
PCB#99	11.8 (9.0–29.8)	10.6 (9.0–12.2)	11.8 (9.6–29.8)
PCB#118	9.0 (5.8–30.6)	7.8 (5.8–21.8)	9.5 (5.8–30.6)
PCB#105	2.7 (1.6–7.4)	3.4 (1.8–6.6)	2.6 (1.6–7.4)
PCB#153	58.8 (29.6–143.8)	45.8 (29.6–117.6)	70.5 (39.2–143.8)
PCB#138	46.4 (23.4–148.4)	32.4 (23.4–79.6)	64.3 (33.2–148.4)
PCB#156	10.6 (3.6–28.0)	8.8 (3.6–26.0)	13.0 (8.2–28)
PCB#157	1.6 (0.8–4.0)	1.4 (0.8–2.8)	2.0 (1.2–4.0)
PCB#180	46.6 (22.0–113.6)	30.6 (22.0–113.6)	55.3 (30.2–71.8)
PCB#170	34.4 (18.6–86.8)	29.4 (18.6–86.8)	45.4 (23.2–77.4)
PCB#189	1.1 (0.6–2.8)	1.2 (0.8–2.8)	1.0 (0.6–2.0)
PCB#194	10.4 (5.2–29.6)	7.6 (5.2–29.6)	12.0 (8.0–21)
PCB#206	2.0 (1.2–5.4)	1.6 (1.2–5.4)	2.2 (1.6–4.0)
PCB#209	3.4 (2.0–6.4)	2.6 (2.0–6.4)	4.1 (2.2–5.6)
p,p'-DDE	37.4 (12.8–215.0)	32.4 (12.8–53.4)	49.2 (16–215.0)
Trans-nonachlor	16.4 (6.4–48.0)	16.2 (6.4–40.6)	16.5 (11.8–48.0)

Values are median (min–max).

2.2. HDL isolation

HDL was isolated from plasma by density ultracentrifugation as described before (Karlsson et al., 2005). In short, plasma was mixed with a sucrose and EDTA solution and overlaid with a KBr/PBS solution with the density 1.063 g/mL. Centrifugation was performed at 290,000g at 15 °C for 4 h in a Beckman Coulter Ti 70.1 rotor. HDL fraction was aspirated and mixed with KBr/phosphate buffer (density 1.24 g/mL) before being subjected to a second round of ultracentrifugation with the same conditions but for 2 h. HDL was collected from the top of the tubes and desalted using PD-10 desalting columns (GE Healthcare, Little Chalfont, UK).

2.3. Sample preparation

Samples were lyophilized and reconstituted in 8 M Urea in 25 mM ammonium bicarbonate. Protein concentrations were measured with 2D Quant Kit (GE Healthcare) before proteins were reduced with dithiothreitol and alkylated with iodoacetamide. Samples were diluted 10× and volume corresponding to 10 µg of total proteins were digested with trypsin (1:25, Promega, Madison, WI, USA) overnight at 37 °C. The following day the peptides were dried with a vacuum centrifugation system and reconstituted in 0.1% formic acid in water.

2.4. nLC-MS/MS

Reconstituted peptides corresponding to 250 ng of proteins were loaded into a nano liquid chromatography system (EASY-nLC, Thermo Scientific, Waltham, MA, USA) with a C18 column (100 mm × 0.75 µm, Agilent Technologies, Santa Clara, CA, USA). Peptides were separated using a 90 min increase from 4% to 40% acetonitrile, supplemented with 0.1% formic acid, followed by an increase to 90% acetonitrile for 10 min. Peptides were analyzed by a data-dependent acquisition method utilizing collision-induced dissociation for sequencing on an LTQ Velos Orbitrap Pro mass spectrometer (Thermo Scientific). Samples were run in triplicate.

Mass spectrometry (MS) data were searched with MaxQuant v1.5.0 (Max Planck Institute of Biochemistry, Martinsried, Germany) utilizing the human Uniprot/Swissprot database (downloaded 20th of August 2014). A mass tolerance of 6 ppm for MS search and 0.6 Da for MS/MS

Download English Version:

<https://daneshyari.com/en/article/5748473>

Download Persian Version:

<https://daneshyari.com/article/5748473>

[Daneshyari.com](https://daneshyari.com)