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ORIGINAL ARTICLE

Reduced high-density lipoprotein 2b in non-obese type 2 diabetic patients analysed by a microfluidic chip method in a case–control study

Xunde Xian[1,2], Yahong Ma[3,4], Danhui D Yang[5], Wei Huang[1], Yuhui Wang[1], Odilo Mueller[5], Elain Chang[5], Yves Konigshofer[5], Mark Van-Cleve[5], Jinkui Yang[3], and George Liu[1]

[1]Institute of Cardiovascular Sciences, Peking University, Beijing, China, [2]Department of Molecular Genetics, UT Southwestern Medical Center, Dallas, TX, USA, [3]Department of Endocrinology, Tongren Hospital, Beijing, China, [4]Department of Endocrinology, Puren Hospital, Beijing, China, and [5]Agilent Technologies, Santa Clara, USA

Abstract

Background: Alterations in high-density lipoprotein (HDL) subfractions, especially in the HDL2b subfraction, have been reported in type 2 diabetes mellitus (T2DM). However, new methods for convenient and reliable quantitation of HDL2b are yet to be developed.

Methods: Thirty-eight patients with T2DM were enrolled and age-, sex- and body mass index (BMI)-matched controls were selected from the same population. A microfluidic chip method was employed to analyse serum HDL subfractions.

Results: The microfluidic chip method revealed a significant reduction in HDL2b and its ratio to total HDL in T2DM patients. There was a reverse correlation for total HDL and HDL2b, and its ratio with triglycerides, homeostasis model assessment-insulin sensitivity index (HOMA-IS) and insulin resistance index (HOMA-IR).

Conclusions: We have shown a reduction of HDL2b and its ratio to total HDL by a novel chip method in T2DM patients. The significant correlation between HDL2b and HOMA-IS and HOMA-IR may have further predictive value in clinical utility.

Keywords: *Type 2 diabetes; HDL subfractions; microfluidic chip; bioanalyser*

Introduction

Patients with type 2 diabetes mellitus (T2DM) often develop dyslipidaemia characterized by increased plasma triglycerides (TG), reduced high-density lipoprotein (HDL) (specifically in the HDL2 subfraction), postprandial lipidaemia and appearance of small, dense low-density lipoprotein (LDL) (Howard et al. 1998). These features are generally considered to be atherogenic and are clinically associated with increased risk of coronary heart disease (CHD) (Haim et al. 1999, Castelli et al. 1977, Watanabe 2006, Zilversmit 1995, Grundy 1997).

Among these alterations in dyslipidaemia, reduced concentrations of HDL subfractions are not well investigated due to limitations in quantitative methods to measure HDL subfractions, such as sequential or gradient ultracentrifugation, gradient gel electrophoresis (Camus et al. 1983, Syvänne et al. 1995), ion mobility measurements, capillary isotachophoresis (Schlenck et al. 1999) and high-performance liquid chromatography (HPLC) (Hughes et al. 1988). Some of these methods are labour intensive, expensive and lengthy; others require expert technical personnel for operation. Although lipoprotein subfraction detection by nuclear magnetic resonance (NMR) technology has been

Address for Correspondence: George Liu, Institute of Cardiovascular Sciences, Peking University and Key Laboratory of Cardiovascular Sciences, Administration of Education, PRC. Tel: +86-10-82802769. Fax: +86-10-82802769. E-mail: vangeorgeliu@gmail.com

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developed in recent years, it has not been widely applied due to complex calculations and instrumental requirement (Otvos et al. 1992).

The novel microfluidic chip-based technique is fast, highly reproducible, easy to operate and has recently been successfully validated and used for HDL2b measurements in subjects recruited in the Prospective Cardiovascular Münster (PROCAM) Study, in which HDL2b was demonstrated to be a new risk factor for cardiovascular disease and can significantly improve the risk prediction of myocardial infarction (Mueller et al. 2008). Based on the previous study using this method we hypothesize that the change of HDL2b level may occur in the population with T2DM and there may be some strong relationships between HDL2b and other cardiovascular risk factors in a T2DM cohort. Here our data demonstrate significant reduction of HDL2b and showed a negative correlation with blood glucose levels, which indicates that HDL2b is one of the potential serum markers for diagnosis of T2DM in the future.

Methods and materials

Study subjects

The study subjects were selected from an ongoing large-scale population-based cohort, the Beijing Community Pre-Diabetes (BCPD) study. A total of 499 T2DM subjects were identified, of whom 285 subjects had previously been diagnosed and the other 214 subjects were identified by oral glucose tolerance test. A total of 1828 subjects were identified as having normal fasting glucose (NFG)

Criteria for participation in the present study included T2DM group in line with 1999 World Health Organization (WHO) diagnostic criteria and NFG group fasting glucose < 5.6 mmol l^{-1}. The exclusion criteria were known history of coronary heart disease, a body mass index (BMI) ≥ 26 kg m^{-2}, BMI < 18 kg m^{-2}, and therapy with insulin, statins, fibrates or thiazolidinediones (TZDs).

Thus, after screening according to the above criteria, only 38 T2DM subjects were recruited for the T2DM group and we randomly selected 38 controls matched by gender and age to the 38 T2DM subjects from 786 NFG cases.

Subjects were taken for laboratory tests and physical examination (measurements of waist circumference, height, weight and blood pressure) performed by a trained nurse. BMI was calculated as weight in kilograms divided by the square of height in metres. The waist to hip ratio (WHR) was also calculated. Fasting serum was obtained and stored at -80°C before biochemical analysis. Fasting blood glucose (FBG), total cholesterol (TC), TG, LDL cholesterol (LDL-c), alanine aminotransferase (ALT) and creatinine (Cr) were determined by the Beijing Tongren Hospital Laboratory with a Beckman Coulter Unicel DX800 (Beckman Coulter Inc., Fullerton, CA, USA). Plasma insulin was measured with an enzyme-linked immunosorbent assay kit made by Linco (Linco Research, St Charles, MO, USA). Homeostasis model assessment–insulin sensitivity index (HOMA-IS) and insulin resistance index (HOMA-IR) were calculated respectively as follows:

$$\text{HOMA-IS} = 20 \times \text{fasting insulin}/(\text{fasting glucose} - 3.5)$$

$$\text{HOMA-IR} = (\text{fasting insulin} \times \text{fasting glucose})/22.5$$

LipoChip analysis

The levels of HDL2b and its ratio to total HDL were measured with two quality control (QC) materials at 0.62 mmol l^{-1} and 1.50 mmol l^{-1} of HDL cholesterol obtained from Solomon Park Research Institute (Kirkland, WA, USA).

All tests were carried out on the Agilent 2100 Bioanalyzer (Agilent, Waldbronn, Germany) using a newly developed HDL subfraction assay as previously described (Meuller et al. 2008). In short, a linear polymer solution of poly(*N*,*N*-dimethyl acrylamide) (PDMA; Polysciences, Warrington, PA, USA) was used as the separation matrix. Serum samples, calibrator and QC materials (Solomon Park Research Institute, Kirkland, WA, USA) were diluted 1:50 in sample buffer (250 mM TAPS, pH 7.5) in the presence of a lipophilic fluorescent dye (Dyomics, Jena, Germany) and allowed to incubate for 5–15 min prior to analysis. Gel wells of the microfluidic chips (Caliper Life Sciences, Hopkinton, MA, USA) were filled with 10 µl of the polymer. The diluted calibrators and QC materials were put in the appropriate wells on the microfluidic chips and patient samples were added to the remaining nine wells. Separation was carried out by starting the chip run, which executed a software program that applied currents and voltages in a predefined manner. Fluorescently stained lipoproteins were detected by laser-induced fluorescence at 680 nm. After completion of the run, the chip was discarded and the electrodes were cleaned with a designated cleaning chip. The entire procedure was carried out in less than 1 h.

Statistical analysis

Normal distributed data were expressed as mean ± standard deviation (SD), while non-normal distributed values (hip circumference, WHR, TC, TG, INS (insulin), HOMA-IS, HOMA-IR, Cr, FBG and ALT) were expressed as median and quartile range (M(QR)).

Some of the non-normal distributed data (TC, TG, HOMA-IS and Cr) were logarithmically transformed for parametric statistical analysis. The significant difference of both normal distributed and transformed parameters between T2DM patients and the controls were assessed with the Student's *t* test. Other sets of data (hip circumference, WHR, FBG, INS, HOMA-IR and ALT), which cannot be normalized, were analysed by the rank sum test. Spearman's rank correlation was used to assess the relationship between HDL, HDL2b, HDL2b/total HDL ratio and other metabolic variables. The statistical calculation was performed by SPSS 11.5 (SPSS Inc., Chicago, IL, USA) and statistical significance was defined as *p*-values less than 0.05 by the two-sided *t*-test.

Results

Clinical characteristics of T2DM and NFG controls

The 38 T2DM patients were fully comparable to the 38 NFG control subjects with regards to sex ratio, age, BMI, WHR and blood pressure, as shown in Table 1.

Laboratory measurements of T2DM and NFG controls

As shown in Table 2, serum levels of FBG, HOMA-IR, TG and TC were significantly higher in T2DM patients than in NFG controls, while HOMA-IS, HDL-c levels were significantly lower. Fasting insulin, LDL-c, ALT and Cr did not differ between the two groups. Other laboratory measurements, such as uric acids and urea nitrogen were also similar in the two groups (data not shown).

Reduced serum HDL2b in T2DM assayed by microfluidic chip method

As a high-throughput and easily operated technique, the microfluidic chip method separates total HDL into several subclasses in less than 1 min, as shown in Figure 1A. Of the subclasses, HDL2b is the one which could be reliably quantified by the chip method (indicated by an arrow). The representative graph from a T2DM patient exhibited a smaller HDL2b peak when compared with that from a NFG control. Quantification of the HDL2b peak revealed that patients with T2DM had significantly reduced HDL2b levels, compared with NFG controls (9.99 ± 3.42 vs 13.82 ± 4.80 mg dl^{-1}, $p < 0.01$, Figure 1B). When expressed as the ratio of HDL2b/total HDL, a similar reduction could be seen in T2DM patients (23.16 ± 6.17 vs $26.58 \pm 6.04\%$, $p < 0.05$, Figure 1C).

Correlations between HDL, HDL2b, HDL2b/total HDL ratio and other metabolic variables

Multiple regression analysis was performed to assess the relationship of HDL, HDL2b and the HDL2b/HDL ratio to some of the metabolic factors which were altered in T2DM. As shown in Table 3, a significant reverse correlation existed for all three HDL-related parameters with serum TGs ($r = -0.47$, -0.59 and -0.62, respectively, $p < 0.01$) and fasting glucose ($r = -0.37$, -0.37 and -0.40, respectively, $p < 0.01$). A low but significant correlation of HDL2b with HOMA-IS (0.23, $p = 0.04$) and with HOMA-IR (-0.24, $p = 0.04$) was observed, while no such relationship existed for total HDL.

Discussion

As suggested by previous studies, distributions of HDL subfractions are widely affected by many factors, such as CHD, dyslipidaemia, obesity and diabetes mellitus (Castelli et al. 1977, Asztalos et al. 2004, Xu & Fu 2003, Tian et al. 2006, MacLean et al. 2000). In this matched case–control study from natural populations we

Table 1. Comparison of clinical characteristics.

Characteristic	NFG	T2DM	*p*-Value
n	38	38	NS
Women (%)	60.5	60.5	NS
Age (years)	56.08 ± 9.87	55.89 ± 9.84	NS
Body height (cm)	160.36 ± 6.85	159.8 ± 7.30	NS
Body weight (kg)	64.46 ± 5.31	63.74 ± 5.85	NS
BMI (kg m^{-2})	25.03 ± 0.28	24.91 ± 0.35	NS
Waist circumference (cm)	84.87 ± 4.16	83.59 ± 4.97	NS
Hip circumference (cm)	96.00 (5.25)	94.00 (4.25)	NS
Waist/hip ratio	0.88 (0.07)	0.90 (0.06)	NS
Systolic blood pressure (mmHg)	133.61 ± 19.61	134.45 ± 21.98	NS
Diastolic blood pressure (mmHg)	81.76 ± 9.21	80.37 ± 11.59	NS

NFG, normal fasting glucose; T2DM, type 2 diabetes mellitus; BMI body mass index. NS, not significant.

Table 2. Comparison of laboratory measurements.

Parameter	NFG	T2DM	p-Value
Fasting glucose (mmol l^{-1})	4.96 (0.57)	6.65 (2.85)	< 0.01
Fasting insulin (pmol l^{-1})	3.31 (3.44)	3.52 (2.76)	NS
HOMA-IS	58.05 (41.23)	19.40 (24.03)	< 0.01
HOMA-IR	0.70 (0.73)	1.20 (1.33)	< 0.01
Triglycerides (mmol l^{-1})	0.96 (0.62)	1.71 (1.93)	< 0.01
HDL cholesterol (mg dl^{-1})	51.39 ± 10.94	42.65 ± 7.62	< 0.01
LDL cholesterol (mmol l^{-1})	2.72 ± 0.78	3.01 ± 0.61	NS
Total cholesterol (mmol l^{-1})	4.40 (1.02)	4.57 (0.84)	NS
Alanine aminotransferase (IU l^{-1})	18.5 (8.25)	24 (10.50)	< 0.05
Serum creatinine (μmol l^{-1})	59 (27.1)	54.5 (24.5)	NS

Parameter values are expressed as mean ± SD and as median and quartile range

NFG, normal fasting glucose; T2DM, type 2 diabetes mellitus; NS, not significant; BMI, body mass index; HOMA-IS, homeostasis model assessment-insulin sensitivity index; IR, insulin resistance index; HDL; high-density lipoprotein; LDL, low-density lipoprotein.

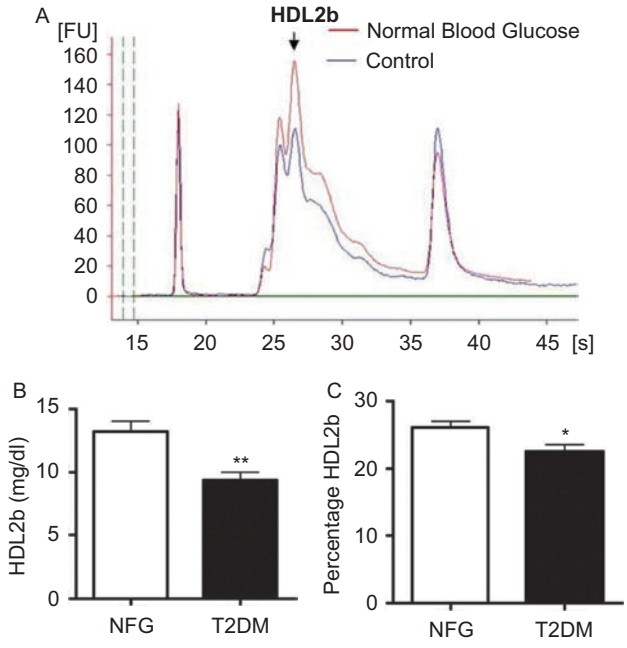

Figure 1. Serum high-density lipoprotein (HDL) subclasses analysed by microfluidic chip. (A) Representative profile of HDL2b indicated by an arrow in the normal fasting glucose (NFG) control (red line) and the type 2 diabetes mellitus (T2DM) patient (blue line). (B) Quantification of HDL2b concentration in NFG controls and T2DM patients. (C) Ratio of HDL2b to total HDL. Data are shown as mean ± SE; $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 3. Multiple regression analysis of high-density lipoprotein (HDL) related to type 2 diabetes mellitus (T2DM).

Metabolic variables	HDL (mg dl^{-1})		HDL2b (mg dl^{-1})		HDL2b/total HDL ratio (%)	
	r	*p*	*r*	*p*	*r*	*p*
Fasting glucose (mmol l^{-1})	-0.37	0.01	-0.37	0.01	-0.40	0.01
HOMA-IS	0.18	0.12	0.23	0.04	0.23	0.04
HOMA-IR	-0.20	0.08	-0.24	0.04	-0.24	0.04
Triglycerides (mmol l^{-1})	-0.47	0.01	-0.59	0.01	-0.62	0.01
Total cholesterol (mmol l^{-1})	0.12	0.30	-0.32	0.01	-0.15	0.19

HOMA-IS, homeostasis model assessment-insulin sensitivity index; IR, insulin resistance index.

studied the alterations of HDL subfraction distribution in a non-obese T2DM cohort, using a microfluidic chip technique. Our results have demonstrated a significant reduction of HDL2b and shown the negative correlation of HDL2b with blood glucose levels and HOMA-IR. These findings are consistent with reports on the HDL subclass in insulin-resistant and T2DM adults from different ethnicities by NMR or native PAGE in which lower levels of large HDL (HDL2b) had been revealed (Garvey et al. 2003, Pérez-Méndez et al. 2007). In our correlation analysis, plasma TG and HOMA-IR correlated inversely with both total HDL and HDL2b, implying that insulin resistance may be responsible for lower levels of large HDLs. However, only HDL2b, but not total HDL, was associated HOMA-IR. Whether this suggests a higher predictable value for HDL2b in T2DM must be specifically addressed in further studies.

Considering that T2DM is a risk factor for CHD and that the larger particle HDL2b is reduced in T2DM, our results further support the hypothesis that large HDL2b particles are more cardioprotective than smaller HDLs through promoting cellular cholesterol efflux, direct antioxidative and anti-inflammatory effects (Lamarche et al. 1997). Paradoxically, small HDL particles have been reported to be better cholesterol acceptors (Davidson et al. 1995), and have enhanced antioxidant properties compared with large HDLs (Kontush et al. 2003, Schiavon et al. 2002). Typically, there are relative or absolute increases in smaller denser HDL3b and HDL3c particles in patients with T2DM, but recently some findings demonstrated that smaller HDL particles did not perform their antioxidative functions in the diabetic condition (Nobécourt et al. 2005), indicating that large-particle HDL2b, but not HDL3, may play a pivotal role in T2DM. Moreover, a number of studies have pointed to the benefit of pharmacological lipid management on CHD risk in T2DM (Downs et al. 1998, Goldberg et al. 1998), and several medications have been used in the treatment of abnormalities of lipoproteins associated with insulin resistance and T2DM, including fibrates, niacin and TZDs (Athyros et al. 2002, Kamanna & Kashyap 2000, Ovalle & Bell 2002). Although most of these drugs increase total HDL concentration in plasma, the subclass profiles of HDLs could not be similarly altered. Our data provide important evidence for future clinical trials using HDL2b as a serum marker for CHD patients with T2DM.

In summary, with the fast and high-throughput microfluidic chip technology, we have shown a reduction of HDL2b in Chinese T2DM patients. The stronger correlation between HDL2b and HOMA-IS/HOMA-IR may have further predictive value in clinical utility.

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***Decleration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista MC, Schaefer EJ. (2004). HDL subpopulations profile and CHD-

prevalence in male participants of the Framingham offspring study. *Arterioscler Thromb Vasc Biol* 24:2181–7.
Athyros VG, Papagergiou AA, Athyrou VV, Demtriadis DS, Kontopoulos AG. (2002). Atorvastatin and micronized fenofibrate alone and in combination in type 2 diabetes with combined hyperlipidemia. *Diabetes Care* 25:1198–202.
Camus MC, Chapman MJ, Forgez P, Laplaud PM. (1983). Distribution and characterization of the serum lipoproteins and apoproteins in the mouse *Mus musculus*. *J Lipid Res* 24:1210–28.
Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. (1977). HDL cholesterol and other lipids in coronary heart disease, The cooperative lipoprotein phenotyping study. *Circulation* 55:767–72.
Davidson WS, Rodrigueza WV, Lund-Katz S, Johnson WJ, Rothblat GH, Phillips MC. (1995). Effects of acceptor particle size on the efflux of cellular free cholesterol. *J Biol Chem* 270:17106–13.
Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM. (1998). Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/ TexCAPS Air Force/ Texas Coronary Atherosclerosis Prevention Study. *JAMA* 279:1615–22.
Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y. (2003). Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 52:453–62.
Goldberg RB, Mellies MJ, Sacks FM, Moyé LA, Howard BV, Howard WJ, Davis BR, Cole TG, Pfeffer MA, Braunwald E. (1998). Cardiovascular events and their reduction with pravastatin in diabetic and glucose intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the Cholesterol And Recurrent Events (CARE) trial. *Circulation* 98:2513–19.
Grundy SM. (1997). Small LDL atherogenic dyslipidemia and the metabolic syndrome. *Circulation* 95:1–4.
Haim M, Benderly M, Brunner D, Behar S, Graff E, Reicher-Reiss H, Goldbourt U. (1999). Elevated serum triglyceride levels and long-term mortality in patients with coronary heart disease: The Bezafibrate Infarction Prevention (BIP) Registry. *Circulation* 100:475–82.
Howard BV, Mayer-Davis EJ, Goff D, Zaccaro DJ, Laws A, Robbins DC, Saad MF, Selby J, Hamman RF, Krauss RM, Haffner SM. (1998). Relationships between insulin resistance and lipoproteins in nondiabetic African Americans Hispanics and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Metabolism* 47:1174–9.
Hughes TA, Moore MA, Neame P, Medley MF, Chung BH. (1988). Rapid quantitative apolipoprotein analysis by gradient ultracentrifugation and reversed-phase high performance liquid chromatography. *J Lipid Res* 29:363–76.
Kamanna VS, Kashyap ML. (2000). Mechanism of action of niacin on lipoprotein metabolism. *Curr Atheroscler Rep* 2:36–46.
Kontush A, Chantepie S, Chapman MJ. (2003). Small dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. *Arterioscler Thromb Vasc Biol* 23:1881–8.
Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. (1997). Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. *Arterioscler Thromb Vasc Biol* 17:1098–105.
MacLean PS, Bower JF, Vadlamudi S, Green T, Barakat HA. (2000). Lipoprotein subpopulation distributions in lean obese and type 2 diabetic women: a comparison of African and white Americans. *Obes Res* 8:62–70.
Mueller O, Chang E, Deng D, Franz T, Jing D, Kincaid R, Konigshofer Y, Kratzmeier M, McNulty M, Qian H, Schneider J, Schulte H, Seedorf U, Tian X, Van Cleve M, Yang D, Assmann G. (2008). PROCAM Study: risk prediction for myocardial infarction using microfluidic high-density lipoprotein (HDL) subfractionation is independent of HDL cholesterol. *Clin Chem Lab Med* 46:490–8.
Nobécourt E, Jacqueminet S, Hansel B, Chantepie S, Grimaldi A, Chapman MJ, Kontush A. (2005). Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia. *Diabetologia* 48:529–38.
Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. (1992). Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single rapid measurement. *Clin Chem* 38:1632–8.
Ovalle F, Bell DS. (2002). Lipoprotein effects of different thiazolidinediones in clinical practice. *Endocr Pract* 8:406–10.
Pérez-Méndez O, Torres-Tamayo M, Posadas-Romero C, Vidaure Garcés V, Carreón-Torres E, Mendoza-Pérez E, Medina Urrutia A, Huesca-Gómez C, Zamora-González J, Aguilar-Herrera B. (2007). Abnormal HDL subclasses distribution in overweight children with insulin resistance or type 2 diabetes mellitus. *Clin Chim Acta* 376:17–22.
Schiavon R, Battaglia P, De Fanti E, Fasolin A, Biasioli S, Targa L, Guidi G. (2002). HDL3-related decreased serum paraoxonase (PON) activity in uremic patients: comparison with the PON1 allele polymorphism. *Clin Chim Acta* 324:39–44.
Schlenck A, Herbeth B, Siest G, Visvikis S. (1999). Characterization and quantification of serum lipoprotein subfractions by capillary isotachophoresis: relationships with lipid apolipoprotein and lipoprotein levels. *J Lipid Res* 40:2125–33.
Syvänne M, Ahola M, Lahdenperä S, Kahri J, Kuusi T, Virtanen KS, Taskinen MR. (1995). High density lipoprotein subfractions in non-insulin-dependent diabetes mellitus and coronary artery disease. *J Lipid Res* 36:573–84.
Tian L, Jia L, Mingde F, Tian Y, Xu Y, Tian H, Yang Y. (2006). Alterations of high density lipoprotein subclasses in obese subjects. *Lipids* 41:789–96.
Watanabe H, Söderlund S, Soro-Paavonen A, Hiukka A, Leinonen E, Alagona C, Salonen R, Tuomainen TP, Ehnholm C, Jauhiainen M, Taskinen MR. (2006). Decreased high-density lipoprotein (HDL) particle size prebeta- and large HDL subspecies concentration in Finnish low-HDL families: relationship with intima-media thickness. *Arterioscler Thromb Vasc Biol* 26:897–902.
Xu YY, Fu MD. (2003). Alterations of HDL subclasses in hyperlipidemia. *Clin Chim Acta* 332:95–102.
Zilversmit DB. (1995). Atherogenic nature of triglycerides postprandial lipidemia and triglyceride-rich remnant lipoproteins. *Clin Chem* 41:153–8.