Lipoprotein Particle Stability in Serum for Nuclear Magnetic Resonance Analysis

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ABSTRACT

Background: Lipoprotein particles consist of cholesterol and other lipids characterized according to their composition, density, size and biological function. It is also known that independent of cholesterol content, lipoprotein particles, including their subclasses, can vary with respect to density, size and lipid content. Associations between the blood concentrations of these particles and increased coronary heart disease (CHD) risk, insulin resistance, diabetes mellitus and metabolic syndrome are well established. Consequently, lipoprotein-lipid profiling may better identify individuals with an increased risk of CHD. Methods for analyzing lipoprotein particles include gel electrophoresis, density gradient ultracentrifugation, ion mobility analysis, and nuclear magnetic resonance (NMR) spectroscopy.

The stability of lipoproteins in biological samples is of importance in clinical settings. The purpose of this study was to assess the stability of lipoprotein particles in serum for analysis by NMR.

Methods: Deidentified residual serum specimens sent to ARUP Laboratories for routine testing were used. Lipoprotein particle concentrations and sizes were measured using the AXINON® lipoFIT® test system incorporating the Bruker Ascend™ 600 Avance™ III HD NMR platform (numares AG, Regensburg, Germany) according to the AXINON test kit protocol. The University of Utah's Institutional Review Board approved this study.

The lipoproteins evaluated were high-density lipoprotein particle number (HDL-p), large high-density lipoprotein particle number (LHDL-p), low-density lipoprotein particle number (LDL-p), small low-density lipoprotein particle number (SLDL-p), large very-low-density lipoprotein particle number (LVLDL-p), high-density lipoprotein particle size (HDL-s), low-density lipoprotein particle size (LDL-s) and very-low-density lipoprotein size (VLDL-s). Conditions included room temperature, refrigerated, frozen (-20 °C) and freeze/thaw cycles.

Results: Summarized in the table below.

Conclusions: Lipoprotein particles HDL-p, LHDL-p, LDL-p, SLDL-p, LVLDL-p, HDL-s, LDL-s and VLDL-s demonstrate reasonable stability in serum at room and refrigerated temperatures for analysis by NMR. However, untimely degradation is possible for LHDL-p, SLDL-p and especially, LVLDL-p for specimens stored frozen at -20 °C.

Lipoprotein Particle Stability

	Room Temperature	Refrigerated	Frozen, -20 °C	Freeze/Thaw
HDL-p	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
LHDL-p	Min 48 hours	Min 30 days	1 Month	3 cycles
LDL-p	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
SLDL-p	Min 48 hours	Min 30 days	2 Months	3 cycles
LVLDL-p	Min 48 hours	Min 30 days	1 Week	2 cycles
HDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
LDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
VLDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles

INTRODUCTION

Lipoprotein particles or lipoproteins, include low-density and high-density forms among others. These particles consist of cholesterol and other lipids, and are categorized according to their composition, size, density and biological function (1, 2). In addition to size and density, lipoprotein particles, including their subclasses or subfractions, can differ widely in regards to apolipoprotein content and other properties independent of cholesterol concentrations (3).

Associations have been well established between the blood concentrations of lipoproteins and increased coronary heart disease (CHD) risk (4–6), insulin resistance (7, 8), diabetes mellitus (9, 10) and metabolic syndrome (4, 11, 12). Because of the worldwide increase in obesity and metabolic syndrome, additional markers for lipoprotein-lipid profiling may aid in better identification individuals with an increased risk of CHD (13–17).

Several methods are available for analyzing lipoproteins. These include gel electrophoresis, density gradient ultracentrifugation and ion mobility analysis (mass spectroscopy). Furthermore, nuclear magnetic resonance (NMR) spectroscopy has been utilized for lipoprotein particle analysis (18–20). Moreover, some studies suggest that lipoprotein particle analysis or lipoprotein profiling by NMR, may be superior to other methods (21–23).

The numares Health AXINON® lipoFIT® test system utilizes NMR technology for assessing lipoprotein particles and their subclasses. Our system incorporates a Bruker Ascend™ 600/Avance™ III HD NMR instrument with the AXINON serum kit and software. Briefly, the kit is used to prepare samples from human serum for NMR spectroscopy analysis by combining the AXINON additives solution with the serum specimen. Additionally, the kit provides calibration and quality control materials for NMR spectroscopic analyses (24).

In the clinical laboratory, the stability of lipoprotein particles in biological samples is of great importance. Because of the diversity in lipoprotein structure (1–3), particle stability between the different classes and subclasses could potentially vary. Therefore, the objective of this study was to evaluate the stability of lipoproteins in human serum for NMR analysis.

MATERIALS AND METHODS

- The numares Health AXINON lipoFIT test system (test kits and software) were acquired from numares AG (Regensburg, Germany).

- NMR measurements were completed using a Bruker Ascend 600/Avance III HD NMR system purchased from Bruker BioSpin Corporation (San Jose, CA).

- Residual serum specimens, sent to ARUP for routine testing, were deidentified using University of Utah Internal Review Board approved protocols.

- Serum lipoprotein particles were measured by NMR according to the test kit manufacturer's protocol, with the mean of duplicate measurements reported.

- Specimens were stored refrigerated short term (≤1 day) or at -70 °C until use.

- Lipoproteins tested were: High-density lipoprotein particle number (HDL-p), large high-density lipoprotein particle number (LHDL-p), low-density lipoprotein particle number (LDL-p), small low-density lipoprotein particle number (SLDL-p), large very-low-density lipoprotein particle number (LVLDL-p), high-density lipoprotein particle size (HDL-s), low-density lipoprotein particle size (LDL-s) and very-low-density lipoprotein size (VLDL-s).

- All lipoproteins were tested using serum pools at two concentration levels, designated as "Level I" and "Level II".

- Designated serum pool aliquots were subjected to the following: Room temperature, 0, 6, 12, 24 and 48 hours; Refrigerated (2–8 °C), 0, 3, 7, 14 and 30 days; Frozen (-20 °C), 0, 1, 2 and 3 months; Freeze/thaw, 0, 1, 2 and 3 cycles.

- Because LVLDL-p was initially found unstable for <1 month frozen, stability was reevaluated in intervals of days.

RESULTS

- **Table 1.** Lipoprotein particle room temperature stability. All lipoproteins studied appear stable for a minimum of 2 days (CVs 0.1–6.4%).

- **Table 2.** Lipoprotein particle refrigerated stability. All lipoproteins studied appear stable for a minimum of 30 days at 2–8 °C (CVs 0.1–6.8%).

- **Table 3A.** Lipoprotein particle frozen stability at -20 °C. Lipoproteins HDL-p, LDL-p, HDL-s, LDL-s and VLDL-s appear stable for a minimum of 3 months (CVs 0.2–5.2%). LHDL-p, SLDL-p and especially LVLDL-p demonstrate unacceptable variance over 3 months (CVs 11.2–24.6%).

- **Table 3B.** Acceptable -20 °C stability intervals for LHDL-p, SLDL-p and LVLDL-p (CVs 1.9–10.6%).

- **Table 4.** Lipoprotein particle freeze/thaw stability. Except for LVLDL-p which is questionable, the lipoproteins studied appear stable for a minimum of 3 freeze/thaw cycles (CVs 0.1–2.9%). LVLDL-p was found acceptably stable for 2 cycles, generating CVs of 6.9 and 1.6% for Levels I and II, respectively.

- **Table 5.** Summary, lipoprotein particle stability.

Table 1. Lipoprotein room temperature stability.

Level	HDL-p (µmol/L) I	II	LHDL-p (µmol/L) I	II	LDL-p (nmol/L) I	II	SLDL-p (nmol/L) I	II	LVLDL-p (nmo/L) I	II	HDL-s (nm) I	II	LDL-s (nm) I	II	VLDL-s (nm) I	II
Hours 0	20.8	25.1	4.6	5.8	864	1342	381	515	3.9	4.2	9.0	9.3	21.3	21.6	46.7	48.9
6	21.3	27.0	5.0	6.4	835	1349	378	511	3.5	4.2	9.0	9.3	21.4	21.6	46.4	48.9
12	21.0	26.1	5.1	6.5	816	1329	361	512	3.5	3.9	9.0	9.4	21.3	21.6	46.5	48.6
24	20.4	24.8	5.3	6.6	804	1297	359	517	3.6	4.0	9.1	9.4	21.3	21.6	46.5	48.8
48	20.1	25.5	5.4	6.9	787	1324	357	495	3.5	3.9	9.1	9.4	21.4	21.7	46.3	48.6
Mean	20.7	25.7	5.0	6.4	821	1328	367	510	3.6	4.0	9.0	9.3	21.3	21.6	46.5	48.8
SD	0.48	0.87	0.32	0.41	29.8	20.0	11.4	8.6	0.18	0.13	0.07	0.07	0.03	0.05	0.15	0.14
CV%	2.3	3.4	6.2	6.4	3.6	1.5	3.1	1.7	5.1	3.1	0.7	0.7	0.1	0.3	0.3	0.3

Table 2. Lipoprotein refrigerated stability, 2–8 °C.

Level	HDL-p (µmol/L) I	II	LHDL-p (µmol/L) I	II	LDL-p (nmol/L) I	II	SLDL-p (nmol/L) I	II	LVLDL-p (nmo/L) I	II	HDL-s (nm) I	II	LDL-s (nm) I	II	VLDL-s (nm) I	II
Days 0	20.8	25.4	4.3	5.6	904	1352	419	527	3.4	5.5	9.0	9.2	21.3	21.6	46.9	48.7
3	20.9	25.9	4.2	5.3	894	1382	429	527	3.3	5.1	8.9	9.15	21.3	21.6	46.3	48.7
7	20.9	24.8	4.1	5.2	888	1340	417	547	3.2	5.0	8.9	9.2	21.3	21.6	46.9	48.6
14	20.8	25.2	4.1	5.3	885	1327	410	536	3.3	4.9	8.9	9.2	21.3	21.6	46.4	48.8
30	20.3	25.0	3.9	5.1	877	1256	424	529	3.2	4.5	8.9	9.2	21.3	21.5	46.0	48.5
Mean	20.7	25.3	4.1	5.3	889	1331	419	533	3.2	5.0	8.9	9.2	21.3	21.6	46.5	48.6
SD	0.25	0.41	0.15	0.19	10.0	47.0	7.2	8.8	0.10	0.34	0.04	0.03	0.02	0.04	0.31	0.20
CV%	1.2	1.6	3.6	3.6	1.1	3.5	1.7	1.6	3.2	6.8	0.4	0.3	0.1	0.2	0.7	0.4

Table 3A. Lipoprotein frozen stability, -20 °C.

Level	HDL-p (µmol/L) I	II	LHDL-p (µmol/L) I	II	LDL-p (nmol/L) I	II	SLDL-p (nmol/L) I	II	LVLDL-p (nmo/L) I	II	HDL-s (nm) I	II	LDL-s (nm) I	II	VLDL-s (nm) I	II
Months 0	28.8	33.1	5.5	12.1	874	1575	359	737	3.4	10.9	8.7	9.8	21.0	22.1	45.6	52.6
1	27.8	34.0	5.8	11.7	882	1615	389	756	2.7	7.6	8.6	9.75	21.2	22.1	45.9	49.2
2	27.5	32.2	4.3	10.6	864	1566	442	728	2.1	7.2	8.7	9.6	21.3	22.1	46.2	49.3
3	25.4	30.5	3.7	9.4	953	1613	480	717	2.1	6.8	8.6	9.5	21.3	22.0	46.1	48.6
Mean	27.4	32.4	4.8	11.0	893	1592	417	734	2.6	8.1	8.7	9.7	21.2	22.1	45.9	49.9
SD	1.41	1.51	1.01	1.23	40.8	25.1	53.9	16.7	0.63	1.88	0.06	0.14	0.15	0.05	0.24	1.83
CV%	5.2	4.6	20.9	11.2	4.6	1.6	12.9	2.3	24.6	23.2	0.7	1.4	0.7	0.2	0.5	3.7

Table 3B. Acceptable frozen stability, -20 °C.

Level	LHDL-p (µmol/L) I	II	SLDL-p (nmol/L) I	II		LVLDL-p (nmo/L) I	II
Months 0	5.5	12.1	359	737	Day 0	4.3	5.9
1	5.8	11.7	389	756	3	4.2	4.9
2			442	728	4	4.1	5.0
3					5	4.1	5.2
					6	4.3	5.1
Mean	5.7	11.9	396	740		4.2	5.2
SD	0.21	0.26	42.0	14.4		0.10	0.38
CV%	3.6	2.2	10.6	1.9		2.4	7.4

Table 4. Lipoprotein Freeze/Thaw Stability.

Level	HDL-p (µmol/L) I	II	LHDL-p (µmol/L) I	II	LDL-p (nmol/L) I	II	SLDL-p (nmol/L) I	II	LVLDL-p (nmo/L) I	II	HDL-s (nm) I	II	LDL-s (nm) I	II	VLDL-s (nm) I	II
Cycles 0	24.0	29.9	5.5	12.1	1082	1567	228	852	5.6	10.9	8.5	10.0	20.5	21.7	45.7	52.8
1	24.1	30.1	5.6	12.0	1072	1532	238	853	5.1	10.6	8.5	9.95	20.6	21.7	45.8	52.4
2	23.5	30.0	5.5	12.2	1037	1509	226	861	4.9	10.6	8.6	10.0	20.6	21.7	45.7	52.2
3	23.9	29.6	5.5	12.3	1016	1503	233	841	4.3	9.2	8.5	10.0	20.6	21.7	45.6	50.7
Mean	23.9	29.9	5.5	12.2	1051	1528	231	851	5.0	10.3	8.5	10.0	20.6	21.7	45.7	52.0
SD	0.27	0.20	0.04	0.13	30.8	28.9	5.5	8.0	0.54	0.76	0.03	0.03	0.05	0.03	0.10	0.90
CV%	1.1	0.7	0.7	1.1	2.9	1.9	2.4	0.9	10.8	7.4	0.3	0.3	0.2	0.1	0.2	1.7

Table 5. Summary, lipoprotein particle stability.

	Room Temperature	Refrigerated	Frozen, -20 °C	Freeze/Thaw
HDL-p	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
LHDL-p	Min 48 hours	Min 30 days	1 Month	3 cycles
LDL-p	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
SLDL-p	Min 48 hours	Min 30 days	2 Months	3 cycles
LVLDL-p	Min 48 hours	Min 30 days	1 Week	2 cycles
HDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
LDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles
VLDL-s	Min 48 hours	Min 30 days	Min 3 Months	3 cycles

CONCLUSIONS

- Lipoproteins HDL-p, LHDL-p, LDL-p, SLDL-p, LVLDL-p, HDL-s, LDL-s and VLDL-s are stable in human serum at room and refrigerated temperatures for minimums of 48 hours and 30 days, respectively.

- Lipoproteins LHDL-p, SLDL-p and especially LVLDL-p exhibit significant variation when stored frozen (-20 °C) for 3 months, with LVLDL-p reasonably stable for only 1 week.

- With the exception of LVLDL-p, lipoprotein particle stability appears acceptable over a minimum of 3 freeze/thaw cycles.

- Overall, we recommend that sera designated for clinical NMR lipoprotein analysis be transported and stored refrigerated. Frozen specimens should be avoided, primarily due to LVLDL-p instability.

ACKNOWLEDGMENTS

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