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 and density. 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 individualize individuals with an increased risk of CHD. Lipoprotein particle analysis using nuclear magnetic resonance (NMR) spectroscopy has been utilized for lipoprotein particle analysis (18–20). Moreover, some studies suggest that lipoprotein particle number and size are independent predictors of CHD risk (13–17).

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. Briefly, the kit is used to prepare samples from human serum for NMR spectroscopy analysis by combining the lipoproteins with a matrix material and analyzing the mixture with NMR. Lipid concentrations were measured using the NMR spectroscopy method, which is based on the chemical shift of the lipid protons. Lipoprotein particle concentrations and sizes were measured using the AXINON™ Lipoprotein Particle Analysis Kit (Bruker BioSpin, Germany) on an Avance III HD NMR instrument (Bruker Biospin, Germany) equipped with a Bruker NMR spectrometer (300 MHz). The kit is used to prepare samples from human serum for NMR spectroscopy analysis by combining the lipoproteins with a matrix material and analyzing the mixture with NMR.

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 content (3).

Several methods are available for analyzing lipoproteins. These include gel electrophoresis, density gradient ultracentrifugation and ion mobility analysis (mass spectrometry). 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 purposes of this study was to evaluate the stability of lipoprotein particles in human serum for NMR analysis.

MATERIALS AND METHODS

The University of Utah Institutional Review Board approved the study. All lipoprotein studies were performed according to the University of Utah Institutional Review Board approved protocols. The University of Utah Institutional Review Board approved protocol for all human studies was obtained.

We would like to thank Taylor Snow and Paula Shelley for specimen collection and deidentification. We also would like to thank Taylor Snow and Paula Shelley for specimen collection and deidentification.

RESULTS

Table 5. Summary, lipoprotein particle stability.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Room Temperature</th>
<th>Refrigeration</th>
<th>Freez/Thaw</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-p</td>
<td>Min 30 days</td>
<td>Min 3 Months</td>
<td>3 cycles</td>
</tr>
<tr>
<td>LDL-p</td>
<td>Min 30 days</td>
<td>Min 3 Months</td>
<td>3 cycles</td>
</tr>
<tr>
<td>VLDL-p</td>
<td>Min 30 days</td>
<td>Min 3 Months</td>
<td>3 cycles</td>
</tr>
<tr>
<td>HDL-s</td>
<td>Min 30 days</td>
<td>Min 3 Months</td>
<td>3 cycles</td>
</tr>
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</tr>
</tbody>
</table>

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

CONCLUSIONS

- Lipoproteins HDL-p, LDL-p, LDL-s, HDL-s and VLDL-s are stable in human serum at room and refrigerated temperatures for minimum of 48 hours and 30 days, respectively.
- With the exception of VLDL-p, lipoprotein particle stability appears acceptable over a minimum of 3 freeze/thaw cycles.

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REFERENCES