High-Density Lipoprotein Apolipoproteins in Urine: II. Enzyme-Linked Immunoassay of Apolipoprotein A-I

Zvenyika A. R. Gomo¹ and L. Omar Henderson²

We have developed a capture antibody, noncompetitive, enzyme-linked immunoassay for urinary apolipoprotein A-I (Apo A-I) in urine, with use of affinity-purified polyclonal antisera against Apo A-I. A 96-well microtiter plate format is used, with unconcentrated urine as sample and dilutions of serum or high-density lipoprotein (HDL) as standards. The intra- and interassay variation (CV) averaged 7.4% and 9.4%, respectively. The limit of detection is low (1.25 ng/L), and no cross-reactivity with Apo B, C, E, or A-II was detected. The mean (±SD) concentrations of Apo A-I in urine of patients with glomerular proteinuria were a thousandfold greater (38.4 ± 23.1 mg/L) than in normal subjects (16.3 ± 11.3 μg/L in men, 17.97 ± 7.7 μg/L in women, a significant difference, P < 0.001). Apo A-I measurements correlated very well (r = 0.92) with selectivity index assessment. The diurnal variation of the concentration of Apo A-I in urine appears to result from dilution related to fluid intake. This enzymatic method is easy to perform, can be used with large numbers of samples, and is adaptable for use in the routine clinical laboratory. The method holds promise for discriminating between normal and subclinical kidney disease populations by measuring the concentrations of urinary Apo A-I excreted on HDL particles.

Additional Keyphrases: reference values · glomerular proteinuria · kidney disease

Plasma proteins excreted by the kidney into the urine are filtered through the kidney glomerulus, which selects proteins by size, charge, and conformation. Filtered proteins and other newly synthesized proteins may be selectively reabsorbed in the tubule. Patients with kidney disease often demonstrate dysfunction in either the glomerular or tubular kidney functions. Proteinuria, an increase in the amount and distribution of proteins seen in the urine, is one such hallmark of kidney disease. Current clinical measures of kidney function include: (a) indicators of glomerular dysfunction, for example, the concentration of creatinine in urine and serum and increased concentrations of urinary albumin; (b) measures of specific proteins assessing tubular function, for example, β2-microglobulin; and (c) assessment of total kidney function via measurement of the glomerular filtration rate, e.g., insulin clearance. Most of these tests, however, are insensitive to early damage, giving abnormal results only when significant pathological kidney dysfunction is present.

Because the spectrum and quantity of protein found in the urine reflects kidney function, analysis of urine for proteins may give clues to minimal or early-stage kidney damage. Concentration of urinary HDL is reported as increased in patients with nephrotic syndromes (1–5). Apo A-I,³ the main protein component of HDL, therefore could be a sensitive indicator of kidney dysfunction, because high-density apolipoproteins reportedly are in low concentration in urine of normal subjects.

Several types of immunoassays have been developed for plasma Apo A-I with use of specific antisera (6). Short et al. (5) reported using an electrommunnoassay to measure urinary apolipoproteins in highly concentrated urine specimens. In this paper we report the development of a sandwich enzyme-linked immunoassay (ELISA) specific for urinary Apo A-I. In the assay 96-well microplates are used, and serum HDL as standard. The method's specificity, sensitivity, and accuracy are defined from results of studies of concentrations of Apo A-I in urine from healthy subjects and from a group of nephrotic patients.

Materials and Methods
Blood and Urine Samples

Twenty-four-hour urine specimens were collected from 50 ostensibly healthy subjects, ages 17–56 years, and 10 adult patients (six men and four women), ages 23–50 years, with biopsy-proven glomerular disease. None of the healthy subjects was taking any drugs during the study. During the 24 h of urine collection, we collected a 10-mL blood sample from each subject, who had fasted overnight. All urine samples were collected at 4 °C without preservatives. Aliquots of serum and urine were stored at 4 °C for immediate use or at −70 °C for subsequent analysis. Timed urine specimens were also collected from healthy volunteers (four men, two women) at different times of the day (first void, 0800, 1200, 1600, and 2000 hours). Fluid intake was recorded during collection of these samples.

Procedures

Isolation of lipoproteins. Very-low-density lipoprotein (VLDL, d = 1.006 kg/L), low-density lipoprotein (LDL, d = 1.006–1.063 kg/L), and high-density lipoprotein (HDL, d = 1.063–1.21 kg/L) were separated from pooled fresh normal serum by ultracentrifugation (7). The HDL fraction was extensively dialyzed against isotonic saline (pH 7.2) containing ethylenediaminetetraacetic acid (EDTA, 0.1 g/L), thimerosal (1.2 g/L), and sodium azide (0.4 g/L) solution. The total protein of HDL was determined by the method of Lowry et al. (8) with bovine serum albumin (Standard Reference Material No. 926; U.S. National Bureau of Standards, Rockville, MD) as standard.

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³ Nonstandard abbreviations: Apo A-I, apolipoprotein A-I; HDL, LDL, VLDL, high-density, low-density, and very-low-density lipoprotein; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.
Apo A-I. Apo A-I was purified as previously described (9). The Apo A-I preparation contained no apolipoprotein A-II, B, C, or E, as determined by gradient electrophoresis on sodium dodecyl sulfate/polyacrylamide gel (3–15%) and by reactivity with specific antisera in the Ouchterlony double-diffusion technique. Its amino acid composition was closely similar to that of purified Apo A-I, as described by Brewer et al. (10).

Antibodies. Antiserum to Apo A-I were raised in goat and rabbit (11). The goat and rabbit anti-Apo A-I IgG were separated with precipitation by ammonium sulfate (12), followed by affinity purification (13). Goat-anti-rabbit IgG labeled with horseradish peroxidase (EC 1.11.1.7) was from Calbiochem, La Jolla, CA.

Immunoassay procedures. We coated the polystyrene microtiter plates (Dynatech Laboratories Inc., Alexandria, VA) with the capture-antibody goat IgG antibody to Apo A-I in carbonate buffer at pH 9.6, 0.2 mL per well, by incubating at 37 °C overnight. The plates were then washed five times in Tris buffer (pH 8.0) containing Tween 20 (polyoxyethylene (20) sorbitan monolaureate) and thimerosal (0.5 mL/L and 0.04 g/L, respectively). Unbound sites on the microtiter plates were blocked with 0.2 mL of pH 7.3 phosphate-buffered isotonic saline (PBS) containing Tween 20, 0.5 mL/L; nonfat dry milk, 10.0 g/L (Carnation Co., Los Angeles, CA); and thimerosal, 0.1 g/L. The plates were then incubated at 37 °C for 2 h, and the wells were then again washed five times with the same Tris buffer. For the dose–response curves, HDL standard was made in PBS–Tween 20 buffer (pH 7.3) containing 0.5 g of bovine serum albumin per liter. The optimum concentration of bovine serum albumin was determined by testing HDL standard in PBS–Tween 20 buffer containing various concentrations of bovine serum albumin: 0.1, 0.125, 0.250, 0.5, 1, 2, and 4 g/L. The HDL standards ranged from 0.25 to 120 ng/200 μL. For assay of Apo A-I in urine of healthy subjects, 0.2 mL of the 24-h urine specimen was pipetted into each well and the plates were incubated at 37 °C for 2 h, followed by the wash procedure as described above. A 1:32,000 dilution of secondary antibody, rabbit anti-Apo A-I IgG, in PBS–Tween 20 containing 1 g of nonfat dry milk per liter was added to each well (0.2 mL per well) and incubated for 2 h at 37 °C, followed by a wash step. We introduced 0.2 mL per well of a 1:40,000 dilution of the tertiary antibody–enzyme conjugate (goat anti-rabbit IgG–horseradish peroxidase) in PBS–Tween 20 and incubated at 37 °C for 0.5 h, then followed this by five washes with Tris buffer. The optimum concentrations of the capture (primary), secondary, and tertiary antibodies were determined by experiments in which increasing dilutions of antibodies and antigen standard were alternately tested against one another.

Measurement of horseradish peroxidase activity. To each well we added 0.2 mL of substrate solution (0.2 g of o-phenylenediamine dihydrochloride) in 100 mL of pH 6.4 phosphate–citrate buffer (KH₂PO₄, 11.4 g/L, citric acid monohydrate 3.28 g/L, and thimerosal 0.1 g/L) containing 50 μL of a 300 g/L solution of hydrogen peroxide. The plates were incubated at 37 °C for 0.5 h. We stopped the reaction with 50 μL of 2 mol/L sulfuric acid, added with a Titertek Autodrop (Flow Laboratory Inc., McLean, VA). Absorbance readings were measured with a Titertek plate reader (Flow Laboratory) at a wavelength of 492 nm. Columns 1 and 12 and rows A and H were not used because of an edge effect, this type of lack of reliability for these plates having been noted in previous studies in our laboratory.

Determination of creatinine in serum and urine and protein in urine. Serum creatinine, urinary creatinine, and urinary protein were measured by unmodified DuPont aca III analyzer methods (14).

Determination of urinary Apo A-I. Urinary Apo A-I was determined in 50 ostensibly healthy volunteers (25 men, 25 women) and in 10 patients with glomerular proteinuria. Diurnal variability in urinary Apo A-I was assessed by measuring Apo A-I in urine specimens collected at different times of the day in a subgroup of three men and women subjects.

Analytical Variables

Linearity and precision. To prepare standard curves we used HDL and purified Apo A-I as standards, the former prepared in different concentrations of Tween 20 (0.05–4 mL/L). Three different urine samples were serially diluted and assayed to assess linearity in dose–response measurements. Intra-assay precision was determined by measuring three different urine samples 30 times in the same assay. To determine interassay variation, three different urines were assayed in triplicate, within daily runs, over several weeks.

Analytical recovery. To assess analytical recovery, we prepared samples containing 10, 20, and 40 mg of purified Apo A-I per liter, with a 24-h pooled urine from normal subjects as the diluent. The 24-h pooled urine and samples containing added Apo A-I were assayed in triplicate within the same run.

Cross-reactivity. Aqueous solutions containing LDL (10, 40, and 312.5 μg/L) or bovine serum albumin (0.125 and 0.5 g/L) were assayed for Apo A-I to determine cross-reactivity.

Selectivity Index

Selectivity index was expressed as the clearance ratio of urinary IgG and transferrin (15). IgG and transferrin were measured by radial immunodiffusion, with use of prepared plates, standards, and controls, according to the manufacturer's protocol (Behring Diagnostics, San Diego, CA).

Results

Validation of Apo A-I ELISA

Figure 1 shows the linearity and similar slopes of the dose–response curves produced with HDL and purified Apo A-I. Apo A-I content of HDL was calibrated against the IUIS Apo A-I and B Reference Material (CDC no. 1883) (16). The proportion of Apo A-I in the HDL fraction was calculated as 85% of total protein, which agrees with other reports (17). Slopes of the dose–response curves were nearly identical. The assay has a linear range from 1.25 to 80 μg/L. All points in the linear range of the dose–response curve were within 95% confidence limits of the common slope for both HDL and Apo A-I. Serum HDL, prepared in a 0.5 g/L solution of bovine serum albumin, was used as secondary standard in subsequent assays, to compensate for protein matrix comparability between standards and urine samples. The results (Figure 2) suggest that such HDL standards can be prepared in bovine serum albumin solutions having concentrations ranging from 0.5 to 4 g/L without affecting assay results, because each concentration of bovine serum albumin within this range yielded dose–response curves with similar slopes.

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maximum immunoreactivity of urinary Apo A-I, we compared the results for HDL measured in different (0.5 to 4 g/L) Tween 20 concentrations (Figure 4). Linear and expected parallel dose–response curves were obtained at all concentrations tested. Results (Table 1) with HDL treated by heating at 52 °C and with detergents were similar to results obtained when only Tween 20 was incorporated in the assay configuration. Complete delipidation with organic solvents (not shown) also did not increase values for measured Apo A-I.

For three urine pools tested, the intra-assay and interassay coefficients of variation (CV) ranged from 6.0 to 8.9%, and 8.7 to 11.6%, respectively (Table 2). Analytical recovery (Table 3) ranged from 96 to 113% and was linearly related to dilution. The assay did not cross-react with LDL, Apo A-II, bovine serum albumin, or human serum albumin, which are contaminants or cross-reacting proteins commonly encountered in Apo A-I assays.

Figure 3 shows the effect of nonfat milk as a preblocker of free sites not taken up by the Apo A-I antibody on the microtiter plates. HDL has been shown to have high affinity for absorption sites on plastic surfaces (1,8), and thus it will react not only with antibody-attached sites, but also with free sites unless they are blocked by a substance such as nonfat milk. Use of nonfat milk at 0.25 g/L did not block the excess binding sites, but concentrations of nonfat milk >0.5 g/L gave consistent and complete blocking of free binding sites on the plates. Prepared plates containing capture antibody, preblocked with nonfat milk, were stored at 4 °C for two weeks or frozen (−70 °C) for periods up to three months without changes in quantitative efficiency or in the slope of dose–response curves.

To determine if the Apo A-I ELISA accurately measures
Table 1. Effects of Delipidation and Denaturing Agents on Determination of Apo A-I in 24 h Urines of Healthy Subjects and Patients with Glomerular Proteinuria*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal subjects (n = 10)</th>
<th>Patients with glomerular proteinuria (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apo A-I concn, µg/L</td>
<td></td>
</tr>
<tr>
<td>Delipidation with Tween 20, 0.5 g/L</td>
<td>17.53 ± 11.23</td>
<td>4.32 ± 4.14 x 10^3</td>
</tr>
<tr>
<td>Heat 3 h at 52 °C</td>
<td>13.54 ± 8.37</td>
<td>(3.55 ± 3.15) x 10^3</td>
</tr>
<tr>
<td>Dissociation with urea, 8 mol/L</td>
<td>18.16 ± 11.75</td>
<td>(4.39 ± 4.05) x 10^3</td>
</tr>
</tbody>
</table>

*Urines were diluted twofold for normal subjects and 500-fold for patients with proteinuria; results, expressed as means ± SD, are corrected for dilution.

<p>| Table 2. Intra- and Interassay Variation of Apolipoprotein A-I Assay |
|-----------------------------------------------|--------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Urine 1</th>
<th>Urine 2</th>
<th>Urine 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay (n = 30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, µg/L</td>
<td>3.89</td>
<td>19.99</td>
</tr>
<tr>
<td>SD, µg/L</td>
<td>0.28</td>
<td>1.19</td>
</tr>
<tr>
<td>CV, %</td>
<td>7.20</td>
<td>5.95</td>
</tr>
<tr>
<td>Interassay, (n = 84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, µg/L</td>
<td>3.70</td>
<td>18.06</td>
</tr>
<tr>
<td>SD, µg/L</td>
<td>0.35</td>
<td>1.57</td>
</tr>
<tr>
<td>CV, %</td>
<td>9.5</td>
<td>8.70</td>
</tr>
</tbody>
</table>

Urinary Apo A-I, Total Protein Loss Per Day, Creatinine Clearance, and Correlation with Selectivity Index

The mean concentrations of Apo A-I in 24-h urine specimens from the ostensibly healthy male subjects were 16.33 (SD 11.29) µg/L (n = 25) and 17.87 (SD 7.70) µg/L (n = 25) in those from the female subjects. No significant sex-related difference in urinary Apo A-I concentration was observed in this sample, but there was wide variability in concentrations within each gender. The average concentration of Apo A-I in the urine of patients (men and women combined) with glomerular proteinuria was 23.10 (SD 38.4) mg/L (n = 10), a thousandfold higher than values found for the urine of the healthy individuals (P <0.001). The mean total urinary protein loss per day was significantly (P <0.001) different (4.90, SD 3.2 g) in the patient group as compared with the 50 healthy subjects (87.06, SD 34.5 mg). The creatinine clearances of the patients ranged from 7 to 46 mL/min, whereas the creatinine clearances of the healthy subjects were between 70 and 120 mL/min (P <0.001). The concentrations of Apo A-I in the timed urine specimens suggest that there was some diurnal variation in urinary Apo A-I concentration (Table 4), it being most concentrated in the morning, first void sample. Other samples obtained during the day contained the expected total amounts of Apo A-I, diluted owing to fluid intake; however, there was wide variability within the subjects sampled.

Calculated selectivity indices in patients with proteinuria ranged from 0.2 to >0.6 and Apo A-I ranged from 8 to 137 mg/24 h. Apo A-I concentrations and selectivity index were highly correlated (n = 13, r = 0.92, P <0.001, y = 0.003x + 0.176).

Proteinuria, characterization of urinary lipoprotein/apolipoproteins, and serum lipid and apo A-I measurements in patients with biopsy-proven nephrosis are described in Part I (19).

Discussion

Enzyme immunoassays, commonly used in clinical laboratories to measure exogenous or endogenous substances that are present in small concentrations, offer several advantages over conventional radioimmunoassay techniques, while retaining the same or superior sensitivity. These advantages include: (a) health and handling problems associated with radioactivity are obviated; (b) shelf life of reagents is longer; (c) there are no separation steps; and (d) one can perform small or large analytical runs with ease. Because our assay includes the use of specific antibodies, avoids concentration and centrifugation steps in the assay, and requires no radioisotopes, it has the potential for widespread application in clinical and research laboratories.

If an assay that measures one component of a macromolecule to infer the mass of the whole molecule is to be useful, there must be a consistent relationship between the marker and the molecule. Our concern that the free protein Apo A-I and not HDL was being filtered by the kidney glomerulus prompted a thorough characterization of urine for the Apo A-I-containing molecule (19). Indeed, urinary Apo A-I is found on a particle with properties similar to native HDL.

Table 3. Accuracy of the ELISA for Apo A-I in Urine

<table>
<thead>
<tr>
<th>Added</th>
<th>Mean observed increase*</th>
<th>SD</th>
<th>Analytical recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.60</td>
<td>2.04</td>
<td>96</td>
</tr>
<tr>
<td>20</td>
<td>20.04</td>
<td>1.85</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>45.32</td>
<td>2.30</td>
<td>113</td>
</tr>
</tbody>
</table>

*Mean of four determinations.

Table 4. Effect of Time of Collection on Apo A-I Concentrations in Urines from Five Healthy Subjects

<table>
<thead>
<tr>
<th>Time</th>
<th>Fluid intake since preceding sample, mL</th>
<th>Apo A-I concn, µg/L</th>
<th>Total Apo A-I loss, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>19.36 ± 9.56</td>
<td>24.48 ± 6.15</td>
<td></td>
</tr>
<tr>
<td>First void</td>
<td>21.50 ± 11.10</td>
<td>6.69 ± 4.54</td>
<td></td>
</tr>
<tr>
<td>0800 hours</td>
<td>410 ± 90</td>
<td>11.50 ± 7.44</td>
<td>1.98 ± 1.50</td>
</tr>
<tr>
<td>1200 hours</td>
<td>372 ± 180</td>
<td>16.90 ± 9.93</td>
<td>2.94 ± 1.94</td>
</tr>
<tr>
<td>1800 hours</td>
<td>245 ± 109</td>
<td>16.86 ± 13.58</td>
<td>1.87 ± 1.16</td>
</tr>
<tr>
<td>2000 hours</td>
<td>396 ± 183</td>
<td>22.47 ± 13.76</td>
<td>1.87 ± 1.21</td>
</tr>
</tbody>
</table>

*All values are mean ± SD of five determinations.

Significantly different (P <0.01) from results for other times.
Therefore, Apo A-I, as a consistent structural component of HDL, reflects the quantity of the lipoprotein in urine.

In the assay, as developed in our laboratory, microtiter plates are used that also are widely used in clinical laboratory studies. The method can be readily adapted for routine measurement in other laboratories, and plates can be prepared in batches and stored frozen until used. The analytical sensitivity of the Apo A-I ELISA allows determination of nanogram amounts of urinary Apo A-I, and normal subjects can be distinguished from individuals with abnormal loss of HDL in their urine (2–5).

The preblocking agent used in an ELISA technique is important, especially when used in analysis for HDL. Vogt et al. (18) demonstrated the high affinity of HDL for plastic surfaces in a model system. The binding affinity of nonfat milk and HDL on the microtiter plate appears to be similar, and both exhibit higher affinity than albumin. Consequently, a high concentration of nonfat milk (20) in proportion to HDL must be used. Indeed, some of the measurement variability observed in this study may be ascribed, in part, to the between-plate variation in the reproducibility of the mass of protein coated on the plate (21). Automation of the ELISA technique should also decrease pipetting error and increase precision.

Assayable Apo A-I has been shown to increase with delipidation of HDL, particularly in RIA methods (22, 23). In this study, we considered pretreating samples to maximize the exposure of antigenic sites. Tween 20 in a concentration of 0.5 g/L appears to maximize the determination of urinary Apo A-I—thus, any lipid-masked urinary Apo A-I antigenic sites (24) are exposed and accessible to antibody, thereby allowing quantitative detection of Apo A-I.

Our results for urinary Apo A-I at different times of the day appear to suggest that a random urine specimen may not be suitable for the assay, because values for urinary Apo A-I in such specimens were lower than those for first-void morning samples, presumably influenced by fluid intake. We recommend that either a 24-h or first-void urine specimen be used for assay of urinary Apo A-I.

HDL was used as standard in the model assay configuration, as a practical consideration, but a calibrated serum pool (25, 26) is a more suitable preparation, because time is saved by eliminating the need for ultracentrifugal preparation of the HDL. Studies in our laboratory have demonstrated parallelism and the same quantitative efficiency when dilutions of a serum-matrix-based preparation (27) were used to generate the standard dose–response calibration curve.

Our method showed no interference from the conditions studied, including urinary protein content or cross-reactivity with other commonly encountered apolipoproteins. This suggests that the Apo A-I ELISA offers a method for use in studying urinary HDL in the early stages of glomerular proteinuria. Short et al. (5), using highly concentrated urine specimens, determined a correlation coefficient of 0.66 between selectivity index (IgG:transferrin) and urinary Apo A-I (mg/24 h). Our correlation coefficient (r = 0.92), measured by use of aliquots of whole urine, covered a similar range of selectivity indices in patients with proteinuria. Our assay system lacked a concentration step, and attendant loss of apolipoprotein, which may explain the improvement in the correlation coefficient in the present study.

Patients with nephrotic syndrome may differ considerably in the etiology of their disease, other aspects of renal function, and treatment modality. They often have increased total and low-density lipoprotein cholesterol in their serum and lower concentrations of HDL (28, 29). The patients examined in the current study lost large amounts of HDL, as measured in terms of Apo A-I, in a 24-h period. This loss of HDL, which can approach or exceed the normal daily rate of synthesis, may therefore decrease the total HDL content of plasma and alter other aspects of lipoprotein metabolism (VLDL and LDL). Alterations in HDL metabolism were confirmed in early lipoprotein-turnover studies (29) in normal adults and in three nephrotic children (4.5 to 15 years old) in whom the biological half-life of HDL in serum was significantly shortened. Although no iodinated HDL was found in the urine of healthy adults, up to 25% of the injected dose was found in the urine in the nephrotic patients. The authors concluded that the nephrotic children had decreased half-lives of HDL for increased fractional catabolic rates.

Alterations in the HDL content of plasma may be qualitative as well as quantitative. In animal-model systems, several stages in the development of nephrosis have been noted in rats treated with adriamycin or puromycin amnucleoside (30, 31). The progression of mild to severe nephrosis in these animals was accompanied by increasing albuminuria, HDL-like particles containing Apo A-I in the urine, and hyperlipoproteinemia. There was a buildup of HDL-1 (d = 1.050–1.090 kg/L) and HDL-2 (d = 1.090–1.21 kg/L) particles in the animals with severe nephrosis. In human nephrotic patients (>3 g/24 h proteinuria), there is an apparent increase, in the urine, of smaller HDL-3 particles (175,000 Da) and a decrease in HDL-2 (5).

The Apo A-I content of urine reflects specific filtration of the glomerular apparatus, along with the degree of proteinuria and measurement of selectivity indices. In a young population of patients with minimal-change glomerular disease, values for Apo A-I in urine correlated highly with total proteinuria (4). In our study we found a thousandfold difference in the urinary Apo A-I content between apparently healthy subjects and patients with documented kidney dysfunction. We think that the urinary Apo A-I assay holds promise for discriminating between normal persons and those with subclinical kidney-disease and should add valuable diagnostic information. Our method is sufficiently sensitive, and it is suitable for use in screening large numbers of urine samples for Apo A-I and for documenting the small increases in HDL concentration in urine that are suggestive of glomerular filtration dysfunction.

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