Urinary Excretion of Some Proteins and Enzymes during Normal Pregnancy

C. K. Cheung, T. Lao, and R. Swaminathan

Total protein (TP), albumin (Alb), transferrin (TRF), retinol-binding protein (RBP), N-acetyl-β-glucosaminidase (NAG), alanine aminopeptidase (AAP), γ-glutamyltransferase (GGT), and creatinine (Cr) were measured in random (untimed) urine samples from 29 nonpregnant women and from pregnant subjects (11 in the first trimester, 34 in the second, and 37 in the third). The excretion of TP, Alb, TRF, NAG, and AAP (relative to creatinine) and the RBP concentration were all higher (P ≤0.05) in the second and third trimesters compared with values for the nonpregnant controls. The GGT/Cr ratio was significantly higher only in the third trimester. The increase in low-molecular-mass proteins and tubular enzymes suggests that at least part of the increase in Alb, TRF, and TP results from decreased tubular reabsorption. We conclude that excretion of both high- and low-molecular-mass proteins is increased during pregnancy.

In normal pregnancy, protein excretion reportedly is increased in the second and third trimesters (1–2). However, there is no agreement as to the contribution of albumin (Alb) to this increase.6 Lopez-Espinoza et al. (3) found Alb excretion to be greatest during the third trimester. Wright et al. (4) found that the Alb/creatinine (Cr) ratio was higher in pregnant subjects than in nonpregnant controls. This was not confirmed by Beetham et al. (5), who found increased fractional clearance of Alb and increased excretion of retinol-binding protein (RBP) in normal pregnancy. Gerô et al. (6) also reported increased excretion of RBP in normal pregnancy.

In view of these discrepant results we measured the concentrations of Alb, RBP, Cr, total protein (TP), transferrin (TRF), and the activities of N-acetyl-β-glucosaminidase (NAG), alanine aminopeptidase (AAP), and γ-glutamyltransferase (GGT) in random (i.e., untimed) urine samples from nonpregnant and pregnant women, and we report our results here.

Materials and Methods

Untimed 20-mL urine samples were collected from 29 nonpregnant women (age range 22–34 y) and 67 pregnant women (11, 34, and 37 in the first, second, and third trimesters, respectively).

The first-trimester subjects were all admitted for therapeutic abortion. Other pregnant subjects, some of whom were studied serially, were all attending the antenatal clinic.

All pregnant subjects had normal blood pressure, normal results for urine culture and microscopy of a mid-stream specimen, and had no glycosuria or proteinuria when tested with the "N-Labtest" reagent strip (A mes, Elkhart, IN). Urine samples were stored frozen at −20 °C until analysis.

Urine creatinine was measured by a standard automated method based on the Jaffé reaction (Astra 8; Beckman Instrument Inc., Palo Alto, CA); the lower limit of detection was 0.9 mmol/L, and the between-assay (n = 20) CV was 2.4% at a concentration of 7 mmol/L. Total protein was measured by the Coomassie Brilliant Blue dye-binding method adapted to a centrifugal analyzer (Cobas Bio; Roche Diagnostics Ltd., Basle, Switzerland). The lower limit of detection was 8 mg/L. Between-assay CVs were 4.5% and 7.6% for TP concentrations of 25.3 and 12.5 mg/L, respectively. Alb, RBP, and TRF were measured immunoturbidimetrically with use of commercial antibodies (Dako, Glostrup, Denmark) and a centrifugal analyzer as described previously (7). The lower limits of detection were 2.5 mg/L, 60 μg/L, and 130 μg/L, respectively. Between-assay CVs were 5%, 15%, and 8.7%, respectively, for concentrations of 13.9 mg/L for Alb, 300 μg/L for RBP, and 460 μg/L for TRF.

Urine NAG, AAP, and GGT were measured spectrophotometrically, based on methods described by Noto et al. (8) and Jung et al. (9, 10). For AAP, the reaction mixture contained, per liter, 2.36 mmol of alanine-4-nitroanilide and 59 mmol of Tris hydrochloride (pH 7.80). For NAG, the reaction mixture contained 3 mmol of sodium N-cresol-sulphonphthaleinyl-N-acetyl-β-D-glucosaminide per liter of 300 mmol/L carbonate buffer (pH 7.45). The reaction mixture for GGT contained 4.8 mmol of γ-glutamyl-4-nitroanilide and 48.8 mmol of glycylglycine per liter of 120 mmol/L Tris hydrochloride buffer (pH 8.2). All enzymes were measured at 37 °C in a centrifugal analyzer (Cobas Bio). The lower limits of detection were 60, 3, and 0.6 U/L for NAG, GGT, and AAP, respectively. Between-assay CVs were 3.4%, 3.6%, and 4% at activities of 663 U/L, 13.3 U/L, and 5.1 U/L, respectively.

Except for RBP, all results are expressed in relation to Cr excretion. RBP results are given as concentrations (μg/L), because many nonpregnant (62%) subjects had urinary RBP concentration below the detection limit of the present assay, making it impossible to calculate the RBP/Cr ratio for all subjects. Results are given as median and range, because the distributions of the data were not gaussian. Groups were compared by Mann–Whitney U test, a P value of ≤0.05 being considered significant, and the relationships between variables were assessed by rank-sum correlation with a commercial computer package (Abstat TM; Anderson-Bell, Canon City, CO).

Results

Table 1 summarizes the urinary excretion of proteins and enzymes by the nonpregnant subjects and pregnant subjects. The 82 pregnant subjects as a group showed a significant increase in all the analytes measured. The TP/Cr ratio for the pregnant women was significantly higher in the second and third trimesters than for the nonpregnant controls. The Alb/Cr and TRF/Cr ratios were

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2 Address correspondence to this author.
3 Nonstandard abbreviations: Alb, albumin; AAP, alanine aminopeptidase (EC 3.4.11.2); Cr, creatinine; GGT, γ-glutamyltransferase (EC 2.3.2.2); NAG, N-acetyl-β-glucosaminidase (EC 3.2.1.30); RBP, retinol-binding protein; TP, total protein; TRF, transferrin.
4 Received March 24, 1989; accepted June 15, 1989.

Table 1. Excretion of Urinary Proteins by Pregnant and Nonpregnant Women (Median and Range)

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant subjects</th>
<th>1st trimester (n = 34)</th>
<th>2nd trimester (n = 37)</th>
<th>3rd trimester (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein, g per mole of Cr</td>
<td>3.14 (1.03-7.6)</td>
<td>4.75 (2.4-12.7)</td>
<td>5.40 (1.1-17.1)</td>
<td>6.29 (1.1-24.9)</td>
</tr>
<tr>
<td>Albumin, g per mole of Cr</td>
<td>1.19 (0.49-2.80)</td>
<td>1.76 (0.37-3.70)</td>
<td>1.94 (0.36-6.85)</td>
<td>1.91 (0.55-7.7)</td>
</tr>
<tr>
<td>Transferin, mg per mole of Cr</td>
<td>42.8 (2.8-58.2)</td>
<td>36.8 (14.5-430)</td>
<td>81.3 (6.7-903)</td>
<td>219.3 (12-55610)</td>
</tr>
<tr>
<td>RBP, µg/L</td>
<td>(&lt;60-840)</td>
<td>(&lt;60-630)</td>
<td>(&lt;60-3740)</td>
<td>(&lt;60-2380)</td>
</tr>
<tr>
<td>Enzymes, kU per mole of Cr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td>16.8 (7.3-49.1)</td>
<td>22.2 (12.0-75.4)</td>
<td>34.2 (14.2-125)</td>
<td>55.4 (10.9-261)</td>
</tr>
<tr>
<td>GGT</td>
<td>4.1</td>
<td>4.6</td>
<td>4.6</td>
<td>4.7</td>
</tr>
<tr>
<td>AAP</td>
<td>0.78 (3.1-7.0)</td>
<td>0.9 (3.1-6.9)</td>
<td>0.9 (0.3-12.5)</td>
<td>1.3 (0.9-25.4)</td>
</tr>
<tr>
<td>TRF/Cr</td>
<td>36.2 (0.44-2.00)</td>
<td>36.2 (0.61-10.9)</td>
<td>0.9 (0.48-2.7)</td>
<td>1.9 (0.19-4.2)</td>
</tr>
</tbody>
</table>

All values are reported in relation to creatinine except RBP, which is given as the concentration. * Significantly different (P < 0.05). ** P < 0.01. *** P < 0.001 from nonpregnant control group by the Mann-Whitney U test. b Observed range (too many "undetectable" results to determine median).

Discussion

In studies on protein excretion, many authors have used timed collection, collection while recumbent, overnight urine collection (11), or 24-h specimens (3). We used untimed urine specimens, because they are conveniently collected and have been shown to be as valid as overnight specimens (12).

Although the proteinuria of pre-eclampsia has been studied extensively (3, 13-17), there are few studies on the excretion of proteins during normal pregnancy. Total protein excretion was found to be increased during normal pregnancy, especially during the third trimester (1, 2). Our results confirm these findings, and in the third trimester the TP/Cr ratio was nearly three times the value for nonpregnant women.

With the introduction of sensitive immunoassay techniques it has been possible to measure the low concentration of urinary albumin in several pathological (11) and physiological states, including pregnancy. Previous studies have reported inconsistent results for albumin excretion during normal pregnancy. Using radioimmunoassay, Lopez-Enpina et al. (3) reported increased 24-h excretion of Alb in normal pregnancy, whereas Wright et al. (4), using a radial-immunodiffusion method, could not demonstrate an increased excretion rate (measured during 2 h) of Alb. The Alb/Cr ratio, however, was increased. This discrepancy was explained by the different protocols for urine collection. However, Beetham et al. (5), using a radioimmunoassay method, did not find an increased urinary Alb/Cr ratio in pregnancy. Our results clearly show that this ratio is increased in the second and third trimesters. The reason for the negative results of Beetham et al. (5) is not clear.

The increased excretion of Alb and TRF could be the result of increased filtration, reduced reabsorption, or a combination of the two. The high glomerular filtration rate during pregnancy (18) probably is a contributing factor. We have previously shown that decreased tubular reabsorption contributes to the albuminuria of diabetes mellitus (19). To assess the contribution of tubular reabsorption, we measured a low-molecular-mass protein—RBP, which is freely filtered and completely reabsorbed by the renal tubules—and some tubular enzymes (NAG, AAP, and GGT). We found that the concentration of RBP in urine increased in the second and third trimester of pregnancy. Other workers have reported increased RBP excretion during pregnancy (5, 6). Urinary excretion of NAG, AAP, and GGT has been used to detect renal tubular dysfunction—as, for example, after renal transplantation (20), the use of nephrotoxic drugs such as cisplatinum and cephalosporins (21), and in diabetes (22). The NAG/Cr, AAP/Cr, and GGT/Cr ratios were all higher in pregnancy, although the increase in GGT was significant only in the third trimester. Increased NAG and AAP during pregnancy have also been reported by others (23). These results suggest that tubular reabsorption of proteins is decreased in normal pregnancy and this may contribute to the observed increase in Alb and TRF excretion.

In contrast to Alb, the TRF/Cr ratio was increased 12-fold in the third trimester and the percentage of "above-normal" TRF/Cr ratios was 83.9% compared with 24.3% for the greatest in the third trimester. In the third trimester, the proportions of subjects with increased ratios were about 30% for TP/Cr, Alb/Cr, and AAP/Cr; 80% for TRF/Cr; and 50% for NAG/Cr.
Alb/Cr ratio. This increased excretion of TRF cannot be explained on the basis of the molecular size, because the relative molecular masses of TRF and Alb are similar (77 000 and 69 000, respectively). However, TRF is less anionic, and therefore we suggest that in late pregnancy there is not only an increase in permeability of the glomerular membrane but also a change in the charge.

We thank the nursing staff of the Obstetrics Unit for helping to collect the specimens and Mrs. Angela Chu for secretarial help.

References


Three Direct Spectrophotometric Methods for Determination of Total Bilirubin in Neonatal and Adult Serum, Adapted to the Technicon RA-1000 Analyzer

Stephen P. Harrison and Ian M. Barlow

We adapted three bichromatic spectrophotometric methods for determining total bilirubin in serum, for use with the Technicon RA-1000 analyzer. The borate buffer (BOR) of Hertz et al. (Scand J Clin Invest 1974;33:215–30), the caffeine buffer (CAF) of Vink et al. (Clin Chem 1988;34:67–70), and the combined borate-caffeine buffer (B-C) of Franzini and Cattozzo (Clin Chem 1987;33:597–9) were compared. All methods required only 10 μL of serum, were precise (between-batch CVs <4.2%, analyte range 64–310 μmol/L), linear to 1000 μmol/L, and insensitive to interference from hemoglobin to 5 g/L. Lipemia, carotene, and methemalbumin interfered positively with each method but insignificantly unless in supranormal concentrations. Only the BOR method was sensitive to protein matrix effect. Neonates' results obtained with all three methods compared well with a Jendrassik-Gröf-based technique. However, samples from adults with cholestasis were overestimated, particularly by the CAF method, but the BOR and B-C methods would be suitable for "stat" bilirubin analysis in these samples.

Accurate, precise measurement of serum bilirubin in neonates is essential for assessing the risk of kernicterus, because the decision for exchange transfusion or phototherapy is based on the actual concentration (1) or rate of increase (2) of bilirubin in serum. Direct spectrophotometry with bilirubinometers and diazo-based methods are probably the more popular assays (3, 4) for measuring bilirubin, although bilirubin-oxidase-based methods (5) are now becoming available.

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