The Effect of Pregnancy on Renal Clearance of Boron in Humans: A Study Based on Normal Dietary Intake of Boron

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The threshold for chronic boron toxicity for humans is not known. Studies by Barr et al. (1993) of human populations living in areas where water supplies have high boron content estimated daily boron intake in the range of 27 mg B/day (0.45 mg B/kg/day). Sayli et al. (1998) studied the fertility rates in Turkish villagers living in close proximity to large borate ore...
bodies and no effects were observed; however, the exact level of exposure was not quantified.

Humans in the United States have an average daily boron intake from diet of about 1 mg B/day (Rainey et al., 1999). Dietary intake values up to 7 mg B/day are reported from Europe (Ploquin, 1967). The element is essential for normal plant growth (Warington, 1923). Its presence in the human diet is largely due to consumption of fruit and vegetables; wine may add 3–4 mg B/day to the diet (ECETOC, 1995).

In order to extrapolate from the large animal boron toxicity database to humans, especially to pregnant women, information on renal clearance of boron was needed. Therefore, this study was designed to measure the renal clearance of boron in pregnant and nonpregnant women consuming a normal diet.

Although there are human populations with a dietary intake of 0.45 mg B/kg/day (Barr et al., 1993), about 27 mg B per day for a 60 kg person, it was decided that the safest way to measure boron renal clearance in pregnant women was to measure the clearance of the boron normally consumed as a constituent of a normal daily diet. This consideration dictated the experimental design used in this study. Since the estimates of dietary intake provided by food frequency questionnaires are of limited accuracy (Bingham, 1997; Bingham et al., 1997; Coates, et al. 1997; Sawaya, et al., 1996), dietary intake of boron was estimated from the total daily renal excretion of boron.

### MATERIALS AND METHODS

**Subjects.** Thirty-two women in good health and without any significant medical disease, who were between the ages of 18 and 40, were recruited for the study. Women with acute illnesses, renal disease, hypertension, diabetes, hematologic, or neuropsychiatric disorders were excluded. Sixteen subjects were primi- or multigravid women with uncomplicated pregnancies in the 2nd trimester (14–28 weeks). Duration of pregnancy was determined by dates provided by the subject and/or primary care physician. Sixteen, nonpregnant subjects were designated as age-matched references. All patients gave written informed consent. This study was approved by the University of California, Irvine (UCI) Human Subjects Review Board.

**Methods.** The source of boron used for the measurement of renal boron clearance was the dietary boron normally present in human food and present in especially high amounts in fruit and vegetables. Renal clearance of boron was measured over 2 time periods, an initial 2-h period and the subsequent 22-h period. Plasma samples and urine samples for boron measurement were obtained and analyzed as indicated below. The resultant plasma concentrations and timed urinary boron excretion amounts were used to calculate clearance, using the relationship that clearance equals the amount of boron excreted in the urine over the measured time interval divided by the average plasma boron concentration over that time interval.

At the beginning of the study, each subject presented to the UCI Division of Nephrology Clinical Research Center. The subject was instructed to empty her bladder and discard the sample. A baseline blood sample was drawn and the patient remained at the Center for the next 2 h, during which time all voided urine specimens were collected. At the end of the 2 h, the patient was asked to empty her bladder; the specimen was collected, the total 2-h urine volume was measured, and the 2-h blood sample was drawn. The subject was given a urine collection container and asked to collect all subsequent urine and return to the Center 22 h later (24 h from baseline). At that time, the 24-h blood sample was drawn.

**Specimens/analytical methods.** At each specified interval, subjects had 6 ml of blood drawn using standard venipuncture technique with a stainless steel, 20–23 gauge needle attached to a plastic syringe. The samples were placed into plastic tubes containing solid lithium heparin, gently rotated twice and centrifuged at 800 rpm for 20 min. Using a plastic pipette, plasma was proportioned into 2 plastic cryovials and frozen at < 20°F until ready for delivery to the analytical laboratories. All urine (2-h and 22-h) was collected in plastic containers and the volumes measured. Aliquots of urine were refrigerated at 40°F in plastic tubes containing no preservatives, until ready for delivery to the analytical laboratories.

Plasma and urine boron were measured at West Coast Analytical Laboratory (WCAS) using inductively coupled plasma-mass spectrometry (ICPMS). All samples were brought to room temperature and either shaken or vortexed to facilitate mixing. After preparing the dilutions, all solutions were also vortexed. The internal standard (IS) used for all samples and standards was 10B at a final concentration of 50 ng/l. Human plasma samples were prepared for testing by making 1/10 v/v dilutions in 0.05% ammonium hydroxide containing 11B internal standard. Urines were diluted 1/10 v/v in 5% nitric acid containing 11B internal standard. Samples were analyzed for 10B and 11B content by inductively coupled plasma-mass spectrometry (ICPMS), using both a VG PlasmaQuad II system and a PE/Sciex Elan 6100 system. The samples were introduced into the ICPMS by utilizing automated flow injection via either a FIAS system or by controlling the time it took the autosampler to fill the sample loop. NIST SRM 951, boric acid, which is certified for both purity and isotopic content, was used to prepare boron standards in the range of 1 μg/l to 200 μg/l. Standards were prepared in both 5% nitric acid containing 10B internal standard and 0.05% ammonium hydroxide containing 11B internal standard. Acid standards were used for acid prepared samples and the basic standards were used for the basic dilutions. Below are typical instrument parameters for data acquisition. Boron background and carryover was controlled by limiting the sample size (approximately 0.3 ml) and concentration (< 200 μg/l). For the VG PlasmaQuad (~0.3 ml), samples were injected using a time-controlled uptake with a Gilson Model 222 autosampler. Acquisition was timed for 10 s in the middle of the 20-s sample-loop plug. The flow rate to the instrument was 1.2 ml/min delivered by a Gilson minimuls 3 peristaltic pump. Data was collected for masses of 11Be, 11B, and 11B using a dwell time of 50 ms for each selected isotope. Washout time was 60 s at 1.2 ml/min. For the ELAN 6100, a 0.2 ml sample loop was used. The sample injection timing was controlled by a PE/SCIEX FIAS 400 unit. A Gilson peristaltic pump at 1.5 ml/min controlled sample flow rates. The flow rate for the FIAS was 1.1 ml/min. Washout time was 15 s at a flow rate of 2.4 ml/min. Acquisition masses were 11Be, 11B, and 11B using a dwell time of 10 ms. All other instrument parameters were typical operating settings recommended by the manufacturers.

All urine and plasma samples were analyzed for creatinine using a quantitative, colorimetric method (Sigma Diagnostics, St. Louis, MO.). Reference samples were obtained from Sigma Diagnostics. In addition, in-house plasma and urine samples were included as reference samples in each batch of samples analyzed. Creatinine clearance was used as a measure of glomerular filtration rate (GFR). The creatinine analyses were performed at the University of California, Irvine.

**Statistics.** All data are expressed as mean and standard deviation. All clearances are normalized to a surface area of 1.73m2 and per kg of body weight. Statistical analysis was done using a two-sample Student’s t-test. The p value was considered to be statistically significant at < 0.05. Outliers were identified from SAS box-plot analysis.
The age, weight, and gestational age of the participants are described in Table 1. Both pregnant and nonpregnant subjects were similarly matched in ages, 26.8 ± 5.9 and 26.7 ± 6 years (mean ± standard deviation), respectively. The mean body weight was slightly higher in the nonpregnant subjects, due to the inclusion of 2 obese but otherwise healthy individuals. All pregnant subjects had a normal intrauterine pregnancy in the second trimester. The mean gestational age was 20.75 ± 5.6 weeks.

Mean serum creatinine was similar in both groups: 0.72 ± 0.07 and 0.76 ± 0.09 mg/dl in pregnant and nonpregnant subjects, respectively (Table 2). Creatinine clearances were similar in pregnant subjects at 2 and 24 h. Nonpregnant subjects had higher creatinine clearances at 2 h when compared with 24 h: 123.0 ± 23.8 ml/min/1.73 m² at 2 h and 101.7 ± 21.8 ml/min/1.73 m² at 24 h (p = 0.03). This was believed to be due to errors resulting from multiple urine collections throughout the day necessary in the 24-h determination. In stable individuals, creatinine production, plasma level, and renal excretion varies little from day to day, and it is known that creatinine production in women is approximately 15 mg/kg of lean body weight per day. In order to further assess the validity of the 24-h collection, daily creatinine excretion was evaluated in our subjects. In pregnant subjects and overweight individuals, a significant portion of weight measured is not lean muscle mass. Hence, 24-h creatinine excretion of >10.5 mg/kg was considered to represent an adequate collection. Individuals with low 24-h, creatinine clearances and urine creatinine excretions of <7 mg/kg/day (C-011, C-013) were excluded from the calculations. They were thought to have submitted an undercollection of urine. Subject P-202 was excluded because the measured creatinine clearance of 64 ml/min/1.73m² was abnormally low. She was a small woman (118 lb), and although her 24-h creatinine excretion was 12 mg/kg, it was believed her pregnancy contributed significantly to her weight, making estimations of lean body mass difficult. It was likely that her low clearance represented an undercollection. Creatinine excretion and creatinine clearance data are depicted in Table 2.

Plasma boron levels at baseline and 2 h were comparable in pregnant and nonpregnant subjects (Table 3). Plasma values measured at 24 h were significantly lower in pregnant subjects when compared with nonpregnant controls (0.013 ± 0.006 μg B/ml and 0.027 ± 0.018 μg B/ml respectively, p < 0.01). There was variability in plasma levels in both groups at all time intervals. Boron excretion in both groups was similar: 1.35 ± 0.64 mg B/day in pregnant subjects and 1.31 ± 0.47 mg B/day in nonpregnant subjects. Boron clearance was not significantly different between pregnant and nonpregnant subjects at 2 and 24 h; however, pregnant subjects tended to have higher clearances.

Comparisons of boron to creatinine clearance or fractional excretion are shown in Table 4. These were calculated by dividing the boron clearance by the creatinine clearance. Both
pregnant and nonpregnant subjects had fractional excretions < 100% at 2 and 24 h. Fractional excretions were not statistically different between groups; however, pregnant subjects tended to have higher fractional excretion of boron.

DISCUSSION

Boron excretion was comparable in the pregnant and nonpregnant subjects and was, respectively, 1.36 (range 0.65 to 2.82) and 1.31 (range 0.68 to 2.29) mg/day. The boron excretion values, together with plasma boron values, were used in the calculation of renal clearance. Boron intake was taken to be equivalent to urinary excretion of boron. The accuracy of estimating boron intake from urinary excretion is based on the findings that systemic absorption of ingested boron in the form of boric acid is rapid and nearly complete, and boron is cleared almost entirely through renal excretion (Hunt et al., 1997; Jansen et al., 1984a,b; Kent and McChance; 1941). In a study in which the dose of boron was measured with great accuracy, 99% of intravenous doses of boron administered as boric acid was recovered in the urine of 8 adult male subjects studied over a period of 5 days by Jansen et al., (1984a). The investigators also concluded that the day-to-day variations in boron excretion in the urine reflect the simultaneous alimentary intake. They found no tendency for boron to accumulate, even in deep compartments.

Plasma boron levels were measured 3 times throughout the day. Mean plasma levels obtained at baseline and 2 h after initiation of the study were comparable between pregnant and nonpregnant subjects. Those obtained at 24 h were significantly lower in the pregnant subjects when compared with nonpregnant individuals. There was substantial variability in plasma values in both groups, undoubtedly the effects of a diet taken ad libitum. It is unclear what biological factor, other than the effects of diet, could result in the difference in plasma levels observed at the 24-h period in the pregnant subjects. The mean plasma boron levels reported in this population of women were comparable to those reported in the literature. Vanhoe et al. (1995), noted blood levels of 0.013 µg B/ml in fasting healthy volunteers, values comparable to those reported in our pregnant subjects at 24 h. Clarke et al. (1987) reported mean blood levels of 0.031 µg B/ml in normal adults, values that were comparable to 0.027 µg B/ml noted in this study’s nonpregnant subjects. As with our experience, both authors noted a significant variability in boron blood levels among the subjects.

Boron clearances were comparable in both groups when measured at 2 and 24 h and whether the clearances were reported by surface area or body weight. However, boron clearances tended to be slightly higher in the pregnant subjects. Clearances were measured at 2 h because this was considered a reasonable period of time for the subjects to remain at the research center. The urine was collected on site in order to insure a complete collection. As expected, boron clearance values for the 2-hr period were comparable to those obtained at 24 h in both pregnant and nonpregnant controls. Renal clearance of boron in all our subjects (pregnant and nonpregnant) was similar to the 60.5 ml/min/1.73 m² reported by Jansen et al., (1984a). The range of the mean boron clearances in all our subjects at 2 and 24 h was from 43.85 to 68.30 ml/min/1.73 m².

In the companion study our laboratory evaluated renal clearances of boron in pregnant and nonpregnant rats fed differing levels of boron in the diet (Vaziri et al., 2001). Boron clearances reported for pregnant animals ingesting a low dose of boron, (0.3 mg/kg) were 29.6 ml/min/1.73 m² or 3.10 ml/min/kg. An interspecies ratio can be calculated by comparing the clearance of rats with the 2hr clearance of pregnant women. Comparisons with the figures reported by surface area reveal an interspecies ratio (rat to human) of 0.43 and by body weight, 3.04. These figures provide workers conducting boron risk assessments with a basis for estimating the toxicokinetic relationship between the rat model and humans. These data should contribute to the calculation of a factor to replace a default uncertainty factor. However, the relationship between boron dose and mean plasma boron levels in the rat and human cannot be compared directly from the data provided by this study and its companion study of the rat (Vaziri et al, 2001), since plasma samples from the rat were delayed for 3 h following administration of the dose in order to insure complete gastrointestinal absorption. Subsequent studies of rats using a constant dose-rate design would allow area-under-the-curve comparisons.

As stated previously, boron is cleared almost entirely through renal excretion (Hunt et al., 1997; Jansen et al., 1984a,b; Kent and McChance; 1941). Therefore, a detailed analysis of boron excretion must include a measure of renal function or glomerular filtration rate (GFR). GFR is ideally measured using a substance that is freely filtered by the glomeruli and neither secreted nor reabsorbed by the renal tubules.

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
<th>t-Test</th>
<th>p-Value</th>
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<tbody>
<tr>
<td>FE (% reported by surface area)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 h</td>
<td>46.7 ± 13.6</td>
<td>57.3 ± 32.3</td>
<td>0.3</td>
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<tr>
<td>24 h</td>
<td>43.2 ± 21.5</td>
<td>62.7 ± 36.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>FE (% reported by body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>44.9 ± 15.1</td>
<td>57.3 ± 32.3</td>
<td>0.2</td>
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</tr>
<tr>
<td>24 h</td>
<td>41.8 ± 21.8</td>
<td>62.7 ± 36.2</td>
<td>0.1</td>
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</table>

Note. Data are reported as mean ± SD. FE, fractional excretion, was calculated by determining the ratio of boron/creatinine clearance × 100. Boron clearances reported by surface area were compared with creatinine clearances reported similarly. Boron clearances reported by body weight were compared with creatinine clearances reported by body weight.

* n = 13; † n = 14; ‡ n = 15; § n = 12.
The most commonly employed biological substance to determine GFR is creatinine. Creatinine clearance was normal in all our subjects and comparable in both pregnant and nonpregnant subjects. Comparison of the clearance of other freely filtered substances, such as boron, with the clearance of creatinine provides insight into the tubular handling of the substance. This ratio, termed the fractional excretion, indicates tubular reabsorption if it is $<100\%$ and tubular secretion if it is $>100\%$ (Brenner et al. 1981). The fractional excretion of boron in all subjects evaluated was $<100\%$ and, therefore, reflected tubular reabsorption in pregnant and nonpregnant subjects. There was no statistically significant difference between the fractional excretion of pregnant and nonpregnant controls. However, there was a trend of increased fractional excretion or reduced tubular reabsorption in the pregnant subjects when compared with nonpregnant subjects. Interestingly, in the rat study, increased fractional excretion of boron in pregnant animals was also observed at all dose levels. The reason for this difference is not readily apparent but may be due to extracellular volume expansion and vasodilation, known features of pregnancy. These physiologic changes inhibit tubular reabsorption by raising peritubular capillary hydrostatic pressures and lowering oncotic pressures (dilutional hypoalbuminemia). Thus, pregnancy-induced physical factors increase boric acid clearance relative to creatinine clearance.

In summary, boron consumption was similar in pregnant and nonpregnant subjects consuming a normal diet. Plasma boron levels were similar in both groups. Renal clearance of boron was comparable in both groups; however, values tended to be slightly higher in pregnant subjects. All subjects evaluated had normal renal function as measured by creatinine clearance. The fractional excretion of boron was $<100\%$ in both groups, indicating net tubular reabsorption. Tubular reabsorption tended to decrease in pregnant subjects.

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REFERENCES


