Increase in Urinary Desmosine and Pyridinoline During Postpartum Involution of the Uterus in Humans (43922)

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Abstract. **One of the most rapid changes in collagen and elastin content of a tissue occurs in the uterus following postpartum involution. We measured the urinary excretion of specific amino acid markers for mature elastin (desmosine [DES] and isodesmosine [IDES]) and fibrillar collagen (hydroxylysyl pyridinoline [HP] and lysyl pyridinoline [LP]) before and after parturition in three gravid subjects. For that purpose, we used an isotope dilution method coupled with gel filtration and HPLC. The highest DES values were found 2–5 weeks postpartum and were 18–45 μg/g creatinine or two to six times those found for healthy neversmoking nongravid females (7.7 ± 0.3 μg/g creatinine, mean ± SE). The highest levels of urinary HP for each subject were found 2–3 weeks after parturition and were 115–607 nmol/mmol creatinine or 4–21 times those found for healthy neversmoking nongravid females (28.1 ± 1.3 nmol/mmol creatinine). For the gravid subjects as a group and also for each subject, the mean values for urinary DES, IDES, HP, and HP/LP during the first 6 weeks postpartum were significantly greater than the mean baseline values beginning 27 weeks postpartum. For the gravid subjects as a group, the mean value for urinary HP/LP during the first 6 weeks postpartum was significantly greater than the value during the 20 weeks preceding parturition. This suggested that the tissue(s) of origin of the excess HP, during the 6 weeks following parturition, was not bony and was consistent with a uterine origin.** [P.S.E.B.M. 1995, Vol 210]

One of the most rapid changes in collagen and elastin content of a tissue occurs in the uterus following postpartum involution (1–3). Morrione and Seifter found a difference of approximately 53 g collagen between the term and the nongravid uterus (1). Woessner and Brewer found approximately 33 g of collagen and 5 g of elastin in the uterus at term compared with 3–7 g of collagen and 0.3–1.8 g elastin in the uterus of nonpregnant women (2). This implied a decrease of 26–30 g of collagen and 3.2–4.7 g elastin in the uterus following parturition. Gunja-Smith and Woessner found a difference between the term and the nongravid uterus of approximately 20 g collagen and 2 g elastin (3).

We have developed an assay for measuring the urinary concentration of specific amino acid markers for mature elastin (desmosine [DES] and isodesmosine [IDES]) and fibrillar collagen (hydroxylysyl pyridinoline [HP] and lysyl pyridinoline [LP]) that uses an isotope dilution method coupled with gel filtration and HPLC (4–6). The crosslink amino acids DES, IDES, HP, and LP are not appreciably metabolized, but are rapidly excreted in the urine intact, reflecting mature elastin and collagen solubilization *in vivo* (7, 8). The purpose of this study was to better understand the relationship among parturition, involution of the uterus, and the appearance of elastin and collagen degradation products in the urine. The present assay was used to measure the excretion of elastin and collagen cross-link amino acids before and after parturition in humans for possible use as a biochemical marker for degradation of uterine elastin and collagen.

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Subjects and Methods

Subjects were either employees of Boston University School of Medicine or family members of employees and were nonsmokers at the time of the study. With informed consent and approval by our institutional review board, spot urine samples were collected during pregnancy and for at least 9 months postpartum. For one subject (Subject 1) 24-hr urine specimens were collected until 6 months postpartum. Samples were frozen and processed within 1 year. Characteristics of the subjects are displayed in Table I.

DES, IDES, HP, and LP Measurements in Urine. Creatinine content was measured using a commercial kit (Sigma Diagnostics, St. Louis, MO). DES, IDES, HP, and LP were measured in urine by our previously described methods (4–6). Aliquots of random urine specimens were spiked with ^{14}C-DES (500 dpm, 1.0×10^4 dpm/nmol) and ^{14}C-HP (300 dpm, 1.1×10^4 dpm/nmol). ^{14}C-DES and ^{14}C-HP were isolated from metabolically labeled neonatal rat aorta smooth muscle cell cultures as previously described (4, 9). The spiked urine samples were subjected to acid hydrolysis, gel filtration, and HPLC. The isotope dilution method is based upon the principle that the decrease in specific radioactivity of the added ^{14}C-DES or ^{14}C-HP is proportional to the amount of endogenous DES or HP present in the sample (i.e., the isotope is diluted by the endogenous DES or HP). DES and IDES values were expressed as μg/g creatinine and HP and LP as nmol/mmol creatinine in accordance with the literature. Urinary excretion of DES, IDES, and HP normalized for creatinine excretion do not differ between 24-hr urine specimens and randomly collected urine specimens from the same individuals (4, 5). LP has not been investigated but is assumed to behave as HP.

Data are presented as the mean ± SD. Comparisons among three groups were made with Statview 4.01 (Abacus Concepts, Berkeley, CA) using the Kruskal-Wallis test. Probability values less than 0.05 were considered significant.

Results and Discussion

Urinary DES and IDES. Peak urinary DES values for Subject 1 and 3 were 24–32 μg/g creatinine on weeks 2 and 5 postpartum, respectively, or three to four times those found for healthy neversmoking nongravid females (7.7 ± 2.0 μg/g creatinine, mean ± SD, n = 11) (Fig. 1A) (6). Subject 2, who delivered twins, exhibited peak urine DES values on week 2 of 45.2 μg/g creatinine or six times the mean for healthy nongravid females. For the subjects, after parturition, the

Table I. Characteristics of Subjects

	Subject 1	Subject 2	Subject 3
Age (years)	24	31	28
Weight (nongravid) (kg)	77.3	90.9	88.6
Height (cm)	160	165	188
Parity[a]	0	1	1
Birth weight (kg)	3.6	3.4 × 2 (twins)	4.2
Lactation present 1 week postpartum	Yes	No	No

[a] Number of previous term pregnancies.

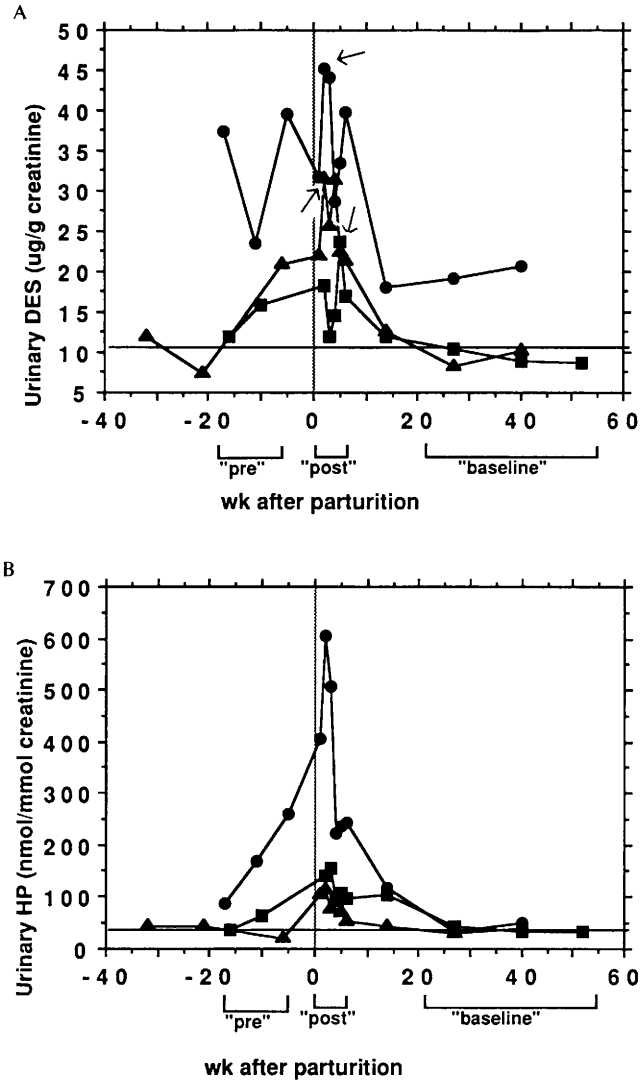

Figure 1. Urinary DES (A) and HP (B) in subjects as a function of time after parturition. The values for Subject 1 are denoted as solid triangles, those for Subject 2 as solid circles, and those for Subject 3 as solid squares. The solid horizontal line represents the mean + 2 SD value (95% confidence limit) for healthy neversmoking nongravid adult females. Peak values of urinary DES for each subject are indicated with an arrow. For each subject, the mean values for urinary DES and HP during the first 6 weeks postpartum ("post") were significantly greater ($P < 0.05$) than the mean of the values for the period beginning 27 weeks postpartum ("baseline"). For Subject 1–3, the DES values (±1 SD) for "post" and "baseline" were 25.7 ± 4.7, n = 6, vs 9.3 ± 1.4, n = 2; 37.1 ± 6.9, n = 6, vs 19.9 ± 1.1, n = 2; and 17.0 ± 4.4, n = 5, vs 9.3 ± 1.0 μg/g creatinine, n = 3, respectively. For Subject 1–3 the HP values were 85.7 ± 22.0, n = 6, vs 35.6 ± 6.4, n = 2, 371.1 ± 162.2, n = 6, vs 41.2 ± 11.5, n = 2, and 119.4 ± 27.0, n = 5, vs 37.6 ± 5.9 nmol/mmol creatinine, n = 3, respectively.

urinary DES values remained for more than 16 weeks above the mean ± 2 SD value (9.7 μg/g creatinine) for healthy neversmoking nongravid females (Fig. 1A). For the gravid subjects as a group and also for each subject, the mean values for urinary DES and IDES during the first 6 weeks postpartum ("post") were more than twice those measured during the baseline ("baseline") period beginning 27 weeks postpartum (27.2 ± 9.8 vs 12.3 ± 5.2 μg/g creatinine, respectively, for DES; and 22.5 ± 9.2 vs 9.7 ± 2.8, respectively, for IDES; $P < 0.05$) (Fig. 1A). In addition, for the gravid subjects as a group, the mean values for urinary DES and IDES during the 20-week period before parturition ("pre") were also more than twice those measured during "baseline" (24.8 ± 11.4 vs 12.3 ± 5.2 μg/g creatinine, respectively, for DES and 19.9 ± 11.8 vs 9.7 ± 2.8, respectively, for IDES $P < 0.05$). The elevation of DES and IDES during the "pre" period may reflect remodeling of fetal elastin. Urinary DES for Subject 2, who remained hypertensive after delivery, was elevated for at least 40 weeks postpartum, approximately 2.5 times the mean for healthy nongravid females.

Urinary HP and LP. The highest levels of urinary HP for Subject 1 and 3 were found 2–3 weeks postpartum and were 115–156 nmol/mmol creatinine on weeks 2 and 3, respectively, or four to six times those found for healthy neversmoking nongravid females (28.1 ± 1.3 nmol/mmol creatinine) (Fig. 1B) (6). Subject 2 exhibited peak urine HP values on week 2 of 606.9 nmol/mmol creatinine or 21 times the mean for healthy nongravid females. After parturition, urinary HP for the three subjects remained above the mean ± 2 SD value (28.1 ± 8.6 nmol/mmol creatinine) for healthy nongravid females for more than 16 weeks (Fig. 1B). For the gravid subjects as a group and also for each subject, the mean values for urinary HP and HP/LP during "post" were significantly greater than during "baseline" (196.4 ± 163.6 vs 38.0 ± 6.8 nmol/mmol creatinine, respectively, for HP; and 9.0 ± 1.8 vs 5.1 ± 1.4, respectively, for HP/LP) (Fig. 1B and 2). In addition, for the gravid subjects as a group the mean value for urinary HP/LP during "post" was also significantly greater than the value during "pre" (9.0 ± 1.8 vs 5.7 ± 1.0, respectively) (Fig. 2). For the gravid subjects as a group, the mean value for urinary LP during "post" was significantly greater than the value during "baseline" (20.7 ± 14.6 vs 7.7 ± 1.7 nmol/mmol creatinine, respectively) (not shown).

Tissue Sources of Elevated HP and LP. We have taken advantage of the tissue specificity of LP to attempt to identify the tissue of origin of the excess collagen degradation. The source of LP is almost exclusively bone, where the ratio of HP to LP is 3.5:1 (8). Although LP is also found in human tendon and articular cartilage, with ratios of HP to LP of 17:1 and 50:1,

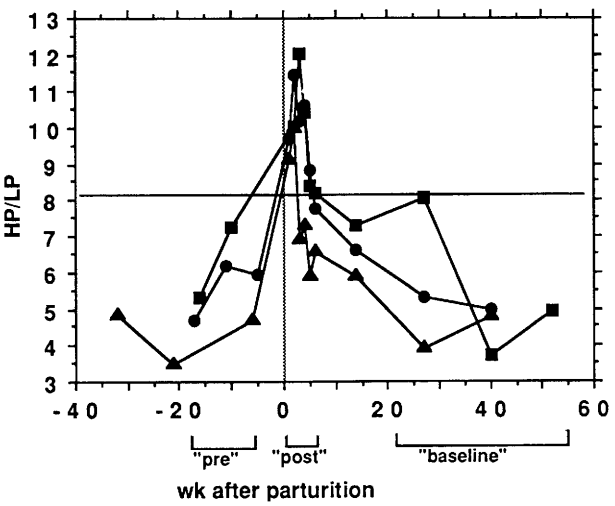

Figure 2. Urinary HP/LP ratio in subjects as a function of time after parturition. The values for Subject 1 are denoted as solid triangles, those for Subject 2 as solid circles, and those for Subject 3 as solid squares. The solid horizontal line represents the mean + 2 SD value (95% confidence limit) for healthy neversmoking nongravid adult females. For each subject, the mean values for HP/LP during the first 6 weeks postpartum ("post") were significantly greater ($P < 0.05$) than the mean of the values for the period beginning 27 weeks postpartum ("baseline"). For Subject 1–3 the HP/LP values (±1 SD) for "post" and "baseline" were 7.6 ± 1.6 $n = 6$, vs 4.3 ± 0.6, $n = 3$; 9.8 ± 1.3, $n = 6$, vs 5.2 ± 0.3, $n = 2$; and 9.8 ± 1.6, $n = 5$, vs 5.6 ± 2.2, $n = 3$, respectively.

respectively, the rate of turnover of collagen in these tissues is usually low and likely contributes little to urinary LP excretion (8). LP is almost undetectable in cow uterus (10). The elevation of HP/LP during the "post" period compared with both the "pre" and "baseline" periods suggested that the tissue(s) of origin of the excess HP, during the 6 weeks following parturition, was not bony, consistent with a uterine origin (see below).

Effect of Pregnancy on Creatinine Excretion. Since the values for urinary DES, IDES, HP, and LP were measured mainly in spot urine samples, creatinine content was used to normalize the values. Variations in creatinine excretion would affect the normalized values. For Subject 1, the mean daily creatinine excretion during pregnancy was 23% greater than during the "post" period (1.65 ± 0.11, $n = 3$, vs 1.34 ± 0.03 g creatinine/day, $n = 6$, respectively, $P < 0.05$). The decrease in creatinine output postpartum, compared with the creatinine output during pregnancy, could account for a portion of the increase seen postpartum in urinary values of DES and HP normalized for creatinine concentration in Subject 1. On the other hand, the absence of fetal-derived DES and HP in the postpartum urine would tend to decrease the amount of urinary DES and HP measured.

Comparison of Urinary Excretion and Uterine Loss of DES and HP. Cumulative excess urinary DES + IDES, above baseline values during the first 6

weeks postpartum, were 3.1, 7.8, and 2.3 μmol for Subject 1, 2, and 3, respectively, implying the degradation of 0.3, 0.7, and 0.2 g elastin, respectively, based upon a composition of elastin in term uterus of 0.94 residues DES + IDES per 1000 (3). This is less than the elastin content difference of 2 g between term and nongravid uterus. The reasons for this discrepancy are not clear, although it has been pointed out that for rats the desmosines cannot be used as a direct measure of uterine elastin content, because of their continuously changing levels (11). With respect to HP, 25 and 38 μmol above baseline were excreted during the first 6 weeks following parturition in Subject 1 and 3, respectively, and 139 μmol HP were excreted by Subject 2. This compares with the difference between term and nongravid uterus of 7–24 μmol HP (3). The subject (Subject 2) who excreted 139 μmol HP and 0.7 g elastin gave birth to twins, and her uterus likely contained larger amounts of elastin and collagen at term. The possible effect of hypertension on urinary DES and IDES in Subject 2 is not known.

Kaidi and coworkers studied urinary excretion of HP in cattle before and after parturition (10). Maximal excretion occurred at Day 6 and returned to baseline values 2–3 weeks after parturition. The rapid return to baseline values in cattle compared with humans may, in part, reflect the lower relative contribution of involution of the uterus to total urinary HP in cattle. In cattle the highest values of HP were three times those of baseline values, compared with 4–21 for humans found in this study (10). Starcher and Percival found that the rat uterus increased in elastin content during pregnancy, reaching levels 300% above nonpregnant controls (12). Elevated levels of urinary DES were also seen during this period. Urinary DES levels reached a maximum 5–6 days after parturition. Within 10 days, postpartum urinary levels had started to return toward control values.

This is the first study in which urinary DES and HP were monitored in humans before and after parturition. Our finding of elevated DES and HP excretion immediately following parturition that peaks 2–5 weeks after parturition is consistent with the sudden decrease in uterine content of collagen and elastin following parturition (3). The enzymes responsible for the proteolysis of uterine elastin and collagen during parturition have been described for rats (13, 14). HP/LP was the only parameter that distinguished the preterm period from the early postpartum period (15–17). The cervix is known to consist of more than 50% collagen by dry weight and lesser amounts of elastin. Collagen destruction appears to accompany the cervical changes of late pregnancy and labor (16). Future studies using more frequent sampling before parturition might indicate whether increases in urinary HP/LP are useful as predictors of parturition.

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