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Quantitation of Urinary Gonadotropins in Normal Children

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ABSTRACT. A simple and improved method for the quantification of urinary LH and FSH was developed. Urinary gonadotropin concentrations were determined by polyclonal double antibody RIA after ammonium sulfate extraction. Urinary LH and FSH concentrated by ammonium sulfate were coeluted with an iodinated LH and FSH tracer. Gel chromatography of the urine revealed that the majority of immunoreactive LH and FSH were eluted coincident with ^{125}I- LH and ^{125}I-FSH. Good correlation was observed between urinary gonadotropin/creatinine ratios in first morning voided and full 24-h urine collections. Age-dependent changes in urinary LH excretion were significant in normal boys and girls 6–17 y of age. Urinary FSH excretion in these children did not change in an age-dependent fashion. (*Pediatr Res* 28: 401–404, 1990)

Abbreviations

FMV, first morning voided

The initial endocrinologic event of puberty is an increase in the amplitude of pulsatile pituitary LH secretion at night secondary to an increase in hypothalamic pulsatile secretion of gonadotropin-releasing hormone (1). However, pulsatile LH secretion has started in prepubertal children (2, 3), but frequent or continuous blood samplings, during the night or over a 24-h period, are necessary to investigate the patterns of pulsatile gonadotropin secretion. This cannot be done easily, especially in normal children. Urinary gonadotropins are thought to reflect the integrated plasma values during the time of collection. It has been reported that FMV urine specimens provide a satisfactory alternative to either serial serum or 24-h urine specimens in children and adult women for studies requiring serial gonadotropin measurements (4–7).

Our purpose was to investigate age-related changes in gonadotropin excretion in normal children. Because FMV urine specimens are easily collected from normal school children at home, we could measure gonadotropins in amounts of urine smaller than those previously reported (8).

SUBJECTS

FMV urine specimens were collected from 480 normal subjects (240 males and 240 females). The subjects were healthy school children 6–17 y of age whose urine specimens were collected for mass screening of renal disorders. The urine specimens with no proteinuria were kindly supplied by the Kanagawa Health Service Association.

FMV and 24-h urine collections were obtained from patients 6–13 y of age whose urine specimens contained no proteinuria during hospitalization in Kanagawa Children's Medical Center. FMV urine specimens were obtained from three healthy children aged 8–13 y and two healthy female adults aged 27–35 y. They were all hospital staff members and their families.

MATERIALS AND METHODS

Urine specimen preparation. Before introduction into the RIA, urinary gonadotropins were extracted with ammonium sulfate. All the urine samples, to which 0.005 vol of 6% sodium azide had been added, were stored at 4°C up to 3 mo until assayed. Four mL of the urine samples were supplemented with 20 μL each of 0.6% BSA and glacial acetic acid, vortex-mixed, and centrifuged at 3000 rpm for 5 min. To 1 mL of the urine supernatant, 0.5 g of powdered ammonium sulfate was added to achieve approximately 75% saturation. The tubes were vortex-mixed, allowed to stand for 30 min at room temperature, and centrifuged at 3000 rpm for 30 min. After aspiration of the supernatant, the pellets were dissolved and neutralized with 100 μL of 0.05 M veronal buffer solution, pH 8.6. Otherwise, extraction procedures were the same as previously reported (9). The urine was concentrated 10-fold for the prepubertal children and 5-fold for the pubertal children.

Immunoassay. Routine measurements of LH and FSH in urine after ammonium sulfate extraction were made by a polyclonal double antibody RIA kit obtained from Eiken Co., Tokyo, Japan. The assay procedure described by the manufacturer was used. LH standard was calibrated WHO 1st IRP 68/40 and FSH standard against the 2nd IRP of pituitary 78/549.

To study the gel chromatographic patterns of immunoreactive LH and FSH in urine, 1 mg of BSA (Armour Pharmaceutical Co., Kankakee, IL) was added to 20 mL of urine from a normal female adult and extracted with ammonium sulfate. The concentrated urine (final volume 1.0 mL) was applied on a 0.95 × 45 cm Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) column and eluted with 0.05 M veronal buffer solution pH 8.6 containing 0.5% BSA. Each fraction was assayed by RIA for LH and FSH. All specimens from a single subject were assayed in the same RIA. To minimize possible error in urine collections and wide variation in body size, immunoreactive LH and FSH in urine were expressed as IU/g urinary creatinine (IU/g Cr). Creatinine concentration in urine was measured by the Jaffe reaction using an autoanalyzer. Differences among mean LH and FSH concentrations of the study groups were detected by analysis of variance using Duncan's multiple range test. Statistical significance for sex differences was determined by the unpaired *t* test. Values are expressed as mean and SD.

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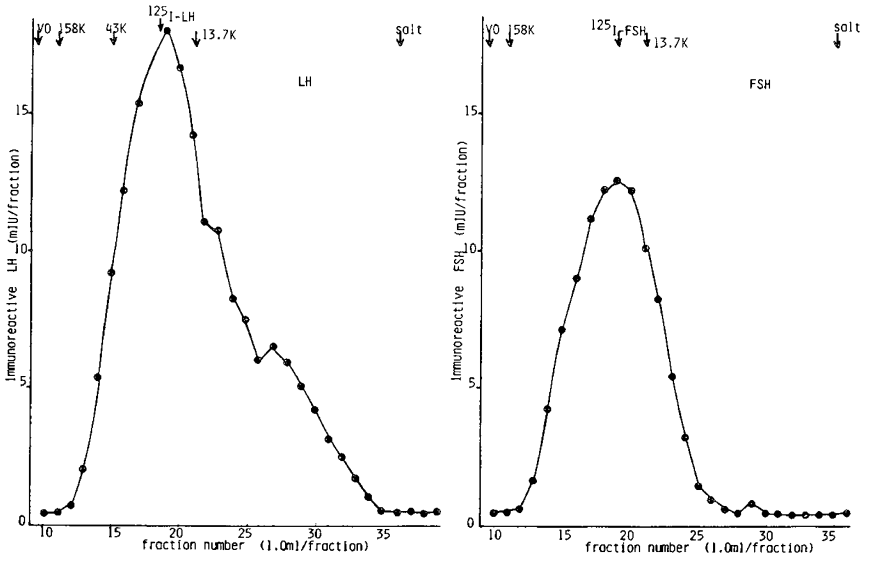

Fig. 1. Gel filtration profiles of immunoreactive LH and FSH in urine from a normal female adult. Twenty mL of the urine sample was concentrated, applied to a 0.95 × 45 cm Sephadex G-100 column, and eluted with 0.05 M veronal buffer solution containing 0.5% BSA, pH 8.6. Each fraction was assayed by RIA for LH and FSH. Calibration was made with blue dextran (*VO*), aldolase (*158K*), ovalbumin (*43K*), ribonuclease A (*13.7K*), ^{125}I-FSH, ^{125}I-LH, and ^{125}I-Na (*salt*).

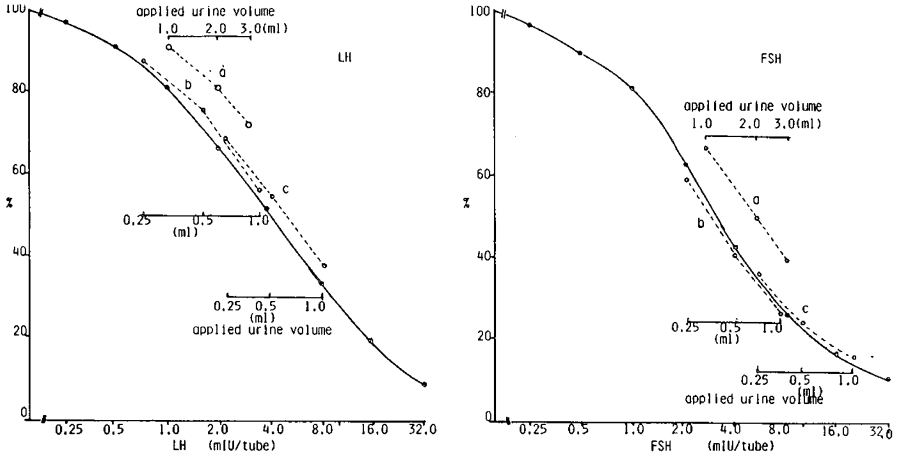

Fig. 2. Standard curve for urinary LH and FSH RIA. Urine samples from three normal children, all family members of the hospital staff, were used for this study. *a*, boy aged 8 y; *b*, girl aged 9 y, *c*, girl aged 13 y.

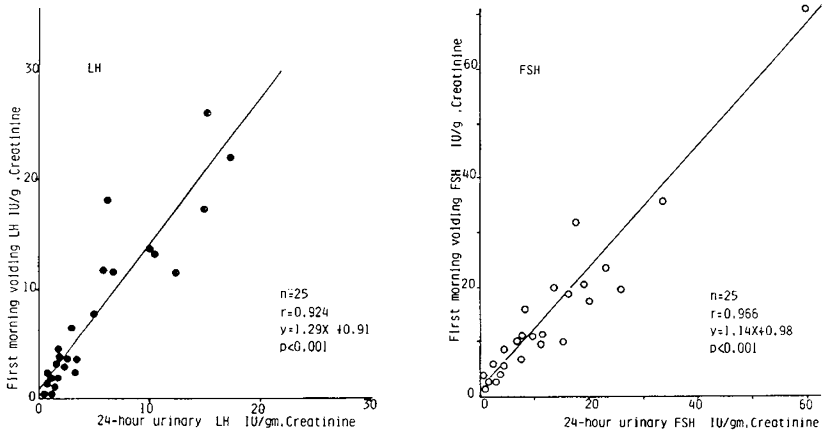

Fig. 3. Correlation between LH and FSH values from FMV and 24-h urine specimens collected from 25 children.

Table 1. *Normal values for urinary LH and FSH according to age and sex**

		Age (y)											
	n	6	7	8	9	10	11	12	13	14	15	16	17
Male													
LH (IU/g Cr)	20	0.77 ±0.54^{f}	1.83 ±2.26^{f}	2.15 ±1.67^{f}	1.95 ±2.53^{f}	7.52 ±4.13^{e}	6.47 ±5.95^{e}	14.67 ±4.68^{d}	22.54 ±8.81^{b,c}	27.68 ±13.33^{a,b}	23.07 ±11.33^{b,c}	26.57 ±10.37^{a,b,c}	22.41 ±11.63^{c}
FSH (IU/g Cr)	20	5.36 ±2.05^{a,b,c,d,e}	6.5 ±4.61^{a,b,c}	11.65 ±5.43	10.08 ±8.04^{a}	11.37 ±7.89	13.48 ±7.66^{e}	11.90 ±4.33^{e}	14.54 ±9.34^{d,e}	14.91 ±11.96^{d,e}	12.58 ±9.10	13.73 ±2.94^{e}	9.36 ±4.67^{a,b}
Female													
LH (IU/g Cr)	20	1.25 ±0.82^{g,h}	1.05 ±0.60^{g,h}	3.43 ±2.07^{g,h}	8.93 ±10.67^{f,g,h}	15.15 ±13.70^{e,f,g}	15.29 ±10.00^{e,f,g}	22.93 ±20.06^{e,f}	44.59 ±24.87^{a,b,c}	47.34 ±22.87^{a,b}	41.03 ±21.43^{a,b,c,d}	33.23 ±20.32^{c,d}	38.07 ±21.53^{b,c,d}
FSH (IU/g Cr)	20	17.70 ±9.03	15.93 ±6.87	22.75 ±12.83	21.65 ±13.66	18.42 ±8.85	20.26 ±15.61	23.55 ±17.79	17.48 ±11.93	13.88 ±6.42	15.86 ±7.38	17.18 ±11.18	16.33 ±9.38

* Values are the mean ± 1 SD obtained from 480 normal urine samples. Any value followed by a superscript differs significantly ($p < 0.05$) from all other values in the same row not followed by the same superscript.

RESULTS

Urinary LH and FSH. The gel chromatographic patterns of immunoreactive LH and FSH are shown in Figure 1. Urinary LH and FSH concentrated by ammonium sulfate were coeluted with an iodinated LH and FSH tracer. Most of immunoreactivity was eluted coincident with the radioactive peak of ^{125}I-FSH and ^{125}I-LH.

In our RIA system for urinary LH and FSH, the binding of ^{125}I-LH and ^{125}I-FSH in the absence of unlabeled hormone was ~40–50% of total radioactivity applied. The sensitivity of the LH and FSH RIA system was 0.25 mIU/tube. About 4.5 mIU/tube of unlabeled LH gave 50% displacement of ^{125}I-LH binding and 3.5 mIU/tube of unlabeled FSH gave 50% displacement of ^{125}I-FSH binding. The recovery of radiolabeled LH and FSH through the extraction was 99.2 ± 0.3 and 98.1 ± 1.2%, respectively (n = 5, mean ± SD). When 4.0 mIU/tube of unlabeled LH and FSH were extracted and measured by RIA, the recovery of immunoreactivity was 97.5 ± 2.5 and 95.1 ± 4.0%, respectively (n = 5, mean ± SD). Parallelism between standard and extracted urine is shown in Figure 2. The intraassay coefficient of variation for the LH and FSH RIA system including the extraction process was ~4.5–9.0%. When urine specimens from a girl aged 11 y stored at 4°C were measured at 2, 4, and 12 wk of storage, the values of LH were 1.3, 1.2, and 1.4 mIU/mL and the values of FSH were 13.3, 13.0, and 13.9 mIU/mL. When urine samples from a boy aged 13 y stored at 4°C were measured at 4, 8, and 12 wk of storage, the values of LH were 21.1, 20.3, and 20.9 mIU/mL and the values of FSH were 16.9, 17.8, and 17.1 mIU/mL. The correlations between urine gonadotropin/creatinine ratios in FMV and 24-h urine collections are shown in Figure 3. The respective correlation coefficients are 0.92 for LH (n = 25) and 0.97 for FSH (n = 25).

Normal values in FMV urinary LH and FSH according to age. Urinary concentrations of LH and FSH from normal subjects of different ages are shown in Table 1.

The concentrations of urinary LH increased slowly until age 12 y, and thereafter increased rapidly in normal boys and girls. Age-dependent changes in urine LH excretion were statistically significant as shown in Table 1. Urinary levels of LH in the girls aged 13–15 y were statistically greater than those for age-matched boys ($p < 0.01$). Urinary FSH excretion increased slightly at the age of 8 y, and thereafter did not change in either sex of children.

DISCUSSION

The gel chromatographic study revealed that the majority of the immunoreactive LH and FSH were eluted with ^{125}I-LH and ^{125}I-FSH, suggesting that most of the urinary materials are monomeric LH and FSH.

Inasmuch as the concentrations of LH and FSH in urine were about 1/10 the corresponding plasma level, and the levels of LH and FSH were low in prepubertal children, the measurement of urinary LH and FSH required extraction and concentration before RIA. The acetone extraction method reported by Kulin *et al.* (5) and Reiter *et al.* (8) would require a large amount of acetone and urine. Ammonium sulfate precipitation is suitable for polypeptides of high molecular weight. We observed that 67–75% saturated ammonium sulfate precipitated ~93–97% of radioactive LH and FSH added to urine. This simple, quick, and inexpensive method of extraction requires a small amount of urine, permits easy extraction of many samples at the same time, consistently provides a high recovery rate, and provides enough sensitivity for LH and FSH determinations in urine. In addition, this method might denature urinary protein less than would previously reported methods using acetone. The ammonium sulfate extraction study with ionic ^{125}I revealed that this step was also effective in removing urinary salts, as reported previously (9). The extraction method involved addition of BSA as well as acetic acid (9). All urine samples were supplemented with BSA

to prevent the adsorption of urinary LH and FSH to the tube walls and to achieve a high extraction rate.

Age-dependent changes in urinary LH excretion were statistically significant in normal boys and girls. The levels of FMV urinary LH and FSH excretion in these boys were comparable with the findings for 24-h urinary excretion of LH and FSH in normal male children reported by Baghdassarian *et al.* (10). Age-dependent changes in urinary LH and FSH excretion in these normal boys and girls were comparable with the findings in the levels of serum LH and FSH in normal children of different ages (11, 12). This method for quantitation of urinary LH and FSH may play an important role in studying the physiology of normal and abnormal puberty.

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