Urinary FSH Glycoform Evaluation by Automated Western Blotting

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FSH is a critical hormone for fertility in women. Its structure and function have been studied for years. In females, FSH enables ovarian follicle development, thereby producing mature oocytes at ovulation. Recently, studies emanating from Dr. Bousfield’s laboratory have shown that human FSH exists as two major glycoforms, fully-glycosylated FSH24, which possesses two α-subunit and two β-subunit asparagine-linked oligosaccharides, and hypo-glycosylated FSH21 or FSH18 glycoforms, which both possess one FSHβ oligosaccharide and two α-subunit oligosaccharides. Pituitary FSH21 abundance exhibits reduced relative abundance with increasing age in women. FSH glycoform concentrations have been reported to vary in serum during the human menstrual cycle. As FSH21 exhibits greater FSH biologic activity than FSH24, age- and cycle-related changes in glycoform abundance may regulate fertility. The goal of this project is to evaluate FSH glycoform ratios in female urine samples. Human FSH and other urinary proteins will be precipitated with ethanol, FSH will be captured by immuno-affinity chromatography using the anti-FSHβ monoclonal antibody, 15-1.E3.E5, followed by gel filtration. Automated Western blotting of 50 ng FSH samples will be used to measure the relative abundance of both FSH glycoforms. First, 200 µg of purified recombinant hFSH were added to ethanol-precipitated urinary proteins and immunocaptured with the 15-1.E3.E5 antibody column. The bound fraction was subjected to Superdex 75 gel filtration and fractions collected by hand. FSH recoveries were measured by an FSH ELISA. In the second series of experiments, 200 ng samples of recombinant hFSH were added to 35-mL samples of precipitated urinary proteins, FSH affinity purified, and FSH quantified by ELISA. Half of the 200-µg added to urinary proteins was bound by the antibody column. Superdex 75 chromatography verified the purity of the FSH and indicated that >80% was heterodimer. When 200 ng FSH samples were added to urinary proteins the column captured most of the FSH, as indicated by the low amounts of FSH immunoactivity in the breakthrough fraction. Most FSH was recovered in the pH 2.7 fraction. Monoclonal antibody 15-1.E3.E5 can capture the majority of 200 ng FSH samples in precipitated urinary protein samples. As automated Western blotting can detect 50 ng FSH samples, it is feasible to measure FSH glycoform abundance in urinary protein samples.