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Rapid fractionation of serum prolactin isoforms by HPLC

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Background: Circulating prolactin exists in a variety of forms, principally monomeric prolactin (23kDa), big prolactin (60kDa) and macroprolactin (150kDa). However, in contrast to monomeric prolactin, big prolactin and macroprolactin are devoid of bioactivity in vivo. To prevent misdiagnosis and mismanagement of hyperprolactinemic patients it is essential to allow for the contribution these high molecular mass bio-inactive forms make to the total serum prolactin concentration. Gel filtration chromatography (GFC) has traditionally been employed as a reference procedure to separate serum prolactin isoforms with subsequent quantitation of the various molecular forms present by immunoassay. However, current GFC methods are slow, labour intensive and not particularly suited to routine use.

Objective: To set-up and validate a rapid, automated and high throughput HPLC method to fractionate serum prolactin isoforms.
Methods: Sera, from healthy individuals, from patients with true hyperprolactinaemia, from individuals with macroprolactinaemia together with a monomeric prolactin standard (National Institute for Biological Standards and Controls, United Kingdom) were subjected to chromatography. Conventional GFC was carried out using an AKTA chromatographic system (Pharmacia Biotech). Serum, 0.2-1.0ml, depending on prolactin concentration, was applied to the column (Superdex SD-75, 60cmx1.6cm) and eluted with TRIS buffered saline, pH 7.4 at a flow rate of 1.0 ml/min. The prolactin content of the fractions, 1.4ml, was measured using an AIA 1800 (Tosoh). For HPLC fractionation, we employed a Waters 1525 system fitted with either a 30cmX0.75cm TSK G3000SW (Tosoh) or BioSep-SEC-3000 (Phenomenex) column. Serum, 0.025ml, applied to the column was eluted with phosphate buffered saline, pH 6.8 at a flow rate of 0.35ml/min. The prolactin content of the fractions, 0.175ml, was measured using an Olympus AU3000i. Both Tosoh and Olympus immunoassays react with all forms of circulating prolactin.

Results: Initial studies revealed that the TSK column was capable of superior fractionation of serum prolactin isoforms in terms of recovery and resolving power relative to the BioSep column. Comparison of the pattern of immunoreactive prolactin isoforms determined by HPLC to that of conventional GFC revealed similar elution profiles. Three main immunoreactive species were identified and corresponded to monomeric prolactin, big prolactin and macroprolactin. The three isoforms were clearly resolved permitting acurate quantitation of the relative contribution of each molecular species to the total serum prolactin concentration. In contrast to a conventional GFC run, which may take up to 120 minutes, the HPLC method can fractionate a sample in under 17 minutes with comparable results. Additional benefits include excellent reproducibility, enhanced sensitivity (<2mU/L) and the ability to fractionate small sample volumes (<100µl).

Conclusions: We have developed and validated a rapid, reproducible and automated HPLC method capable of separating serum prolactin isoforms with greatly enhanced sensitivity and throughput. This methodology will facilitate more precise and specific quantitation of the biologically active and clinically relevant 23kDa monomeric form of prolactin in serum.