

## **Gonadotropin Glyco-fingerprinting by MALDI-TOF mass spectrometry.**

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Up to 30% of gonadotropin mass is sugar and the N-linked glycosylations of the alpha and beta subunits of these hormones are extremely large moieties; obscuring extensive areas of amino-acid backbone of the protein structure. Alteration in glycosylation structure is a known feature of many disease processes. Most notable is hCG hyperglycosylation with respect to choriocarcinoma and Down syndrome. Modern mass spectrometry is making analysis of glycosylation easier, but the traditional approach of cutting of the sugars and then subjecting the resultant extracts to analysis, such as MS, is not necessarily informative. We have adopted a glyco-fingerprint approach whereby the mass of the gonadotropin, with glycosylation remaining intact and in situ, is reduced to the high resolution m/z range of the mass analyser. A spectral finger print is produced whereby the multiple peptide linked glycovariants are resolved. Simple mathematical subtraction of the known peptide sequence from the mass of the resolved glycan-peptide peaks gives the mass of the incumbent glycosylation and by deduction its structure. This reveals protein glycosylation structural information, not just the compositional analysis of the total sugar moieties extracted from the sample. This is a very simple procedure with respect to analysis of urinary core gonadotropin fragments such as hCG $\beta$ cf and LH $\beta$ cf. Indeed, peaks indicative of hyper-glycosylation are easily identified and, in the case of LH $\beta$ cf, a genetic mutation leading to an additional site of N-linked glycosylations is easily demonstrated. Thus, patterns or glyco-fingerprints, of peptide glycosylation may be associated with specific disease processes. This approach may prove more useful as, not only can you detect quantitative and qualitative changes in glycosylation; its ease of procedure lends itself to routine clinical monitoring technologies.