# Abstract

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## The role of the asparagine-linked oligosaccharides of the alpha-subunit in human thyrotropin bioactivity.

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**OBJECTIVE**: TSH and the gonadotropins (FSH, LH, and hCG) are a family of heterodimeric proteins that share a common alpha-subunit and differ in their hormone-specific beta-subunit. The asparagine-linked (N-linked) oligosaccharides on these hormones are important in signal transduction. The N-linked oligosaccharides on the alpha-subunit have no effect on hCG and hFSH receptor binding, but are critical for their biological activity.

**METHODS**: Here, we analyzed the role of alpha-subunit N-linked oligosaccharides in human TSH (hTSH) bioactivity by site-directed mutagenesis and gene transfer. This was achieved by mutating the asparagine (Asn) residue in the N-linked glycosylation consensus sequence (Asn-X-Thr/Ser) to aspartic acid. The wild-type hTSH and its variants were expressed in Chinese hamster ovary cells. Wild-type alpha-subunit and its mutants (alpha 1, alpha 2, and alpha(1 + 2)) were efficiently combined with TSH beta-subunit and secreted as dimers. The bioactivity of TSH glycosylation variants was determined by measuring their abilities to stimulate cAMP formation and T3 secretion using a serum-free culture system of human thyroid follicles.

**RESULTS**: Using this system, wild-type hTSH was significantly effective in the stimulation of cAMP formation and T3 secretion. Deletion of the oligosaccharide units from either site 1(alpha 1) or site 2(alpha 2) of the alpha-subunit increased the biological activity of the dimer by about 30%. However, deletion of carbohydrate units from both sites of hTSH alpha-subunit (alpha(1 + 2) resulted in a significant reduction in cAMP formation (by approximately 70%) and T3 secretion (by approximately 40%) compared to that with wild-type hTSH.

**CONCLUSIONS**: These findings emphasize the importance of the alpha-subunit N-linked oligosaccharide chains on hTSH bioactivity.

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