# THE UTILITY OF FT4 SERUM IN NEWBORNS AT RISK FOR CONGENITAL HYPOTHYROIDISM (CH)

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**Abstract.** Thyrotropin (TSH) stimulates hormonogenesis using the iodate substrate and tyrosine amino acid. After various enzymatic reactions, thyroxine (T4) and triiodothyronine (T3) are released. Part of the hormone freely circulates in serum as free T3 (FT3) and free T4 (FT4). TSH is released after feedback. A study was undertaken to determine cut-off levels of FT4 to verify hypothalamic and pitutitary hypothyroidism. Both TSH and T4 were determined on blood spots by chemoluminescence and radioimmunology. TSH, T4 and FT4 were determined in control cases using an immuno-luminometric method. All newborns with TSH>3 mIU/l and FT4<0.6 ng/l were followed. Some of the positive cases may have resulted from iodine deficiency as a result of geography and environment. New risk values are being evaluated based on spot testing on the third day of blood sample collection. The ongoing study of new cut-off values in relation to birth day and the introduction of FT4 control serum level seems to prevent repeated measurements and promote quicker intervention by physicians thus preventing difficult genetic investigations.

## INTRODUCTION

The thyroid gland synthesizes, stores, and secretes thyroid hormones, mainly l-thyroxine (T4). L-thyroxine circulates predominantly bound to specific serum binding proteins. Although <0.1% of circulating T4 is in the free form [free T4 (FT4)], it is the FT4 concentration rather than the total serum T4 that reflects thyroidal activity. L-triiodothyronine (T3) is produced from T4 by deiodination, mainly in the liver, kidney and muscle; at least 80% of the serum T3 is derived from this metabolic pathway. Since the enzymatic pathways that deiodinate T4 to produce T3 are regulated, in part, by factors that are independent of thyroid function, measurements of serum T3 are not generally required to assess thyroid function. Measurement of serum T4 and FT4 estimate should be the principal measurements of thyroid gland secretion.

The thyroid gland cannot function normally unless it is exposed to thyrotropin or thyroid stimulating hormone (TSH), produced by the thyrotrophs of the anterior pituitary. TSH in serum binds to specific TSH receptors, membrane proteins that are present in all thyroid follicular cells. When TSH binds to its receptors, the activity of adenylate cyclase is increased, resulting in an increase in intracellular cyclic adenosine monophosphate (CAMP) and stimulation of thyroid hormone synthesis and secretion. Serum thyroid hormones, in turn, regulate synthesis and secretion of

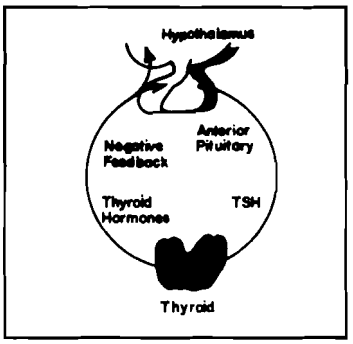


Fig 1. Normal hypothalamic-pituitary-thyroid axis.

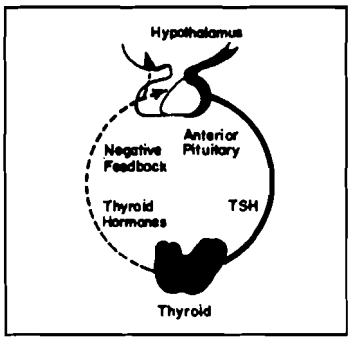


Fig 2. Hypothalamus-pituitary-axis in primary hypothyroidism.

TSH in a negative-feedback manner (Fig 1). When serum thyroid hormone concentrations are decreased because of thyroid disease, to below an individual's threshold for thyroid hormone sufficiency, serum TSH concentration increases. The aim of this project was to determine the serum level of FT4 to verify hypothalamic and pituitary hypothyroidism.

## MATERIALS AND METHODS

Both TSH and T4 on blood spots were determined by chemoluminescence and radioimmunology with cut-off values of >20 mIU/l and <2.5 ng/dl respectively depending on method sensitivity. TSH, T4 and FT4 were tested using the immunoluminometric method on the LIAISON immunoassay analyzer, a fully automated random access analyzer using magnetic particle technology for separation and isoluminol chemoluminescence of the immunocomplex (Hubl *et al*, 2000). TSH was measured from a 200 µl serum sample using a one-step anti-TSH β-chain immunoassay covering a concentration range from 0.004 to 100 mIU/l. The competitive FT4 assay used the Spalt principle with an isoluminol labelled monoclonal antibody as tracer. Immune extraction of labelled antibodies not bound by endogenous antigen from samples was performed using an excess of covalently immobilised T4 antigen on the solid phase.

## RESULTS

All newborns with serum TSH of >3mIU/l and FT4 <0.6 ng/dl levels were sent for follow-up. Most patients had primary hypothyroidism and were readily diagnosed by finding a subnormal serum concentration of FT4 in association with an increased serum TSH concentration. Since hypothyroidism occasionally develops in patients with normal thyroid glands because of inadequate stimulation by TSH resulting from disorders of the anterior pituitary or hypothalamus, diagnosis was confirmed by finding subnormal serum FT4 concentration in association with either normal or decreased serum TSH. This diagnosis, although uncommon, was extremely important because TSH deficiency may coexist with significant deficiencies of other anterior pituitary hormones, particulary corticotropin (ACTH) (Surks and Ocampo, 1996).

## DISCUSSION

Neonatal screening for primary hypothyroidism that relies on a single measurement of TSH fails to detect any cases of congenital disease in which TSH concentrations are not raised. Thyroid hormone deficiency, occurring in uterus, or at any stage during the critical developmental period, leads to impaired growth and neuropsychological development of the child. Therefore, careful investigations of all suspected cases of neonatal hypothyroidism are important, especially when TSH concentrations appear normal.

A novel form of hypothyroidism resulting from pituitary thyroid-feedback hypersensivity (PTFH) has been identified (Frankton *et al*, 2000). PTFH cannot be detected by a TSH screening test and is characterised by moderate, long-term peripheral thyroid hormone deprivation in the presence of normal circulating TSH. In our patient, the disorder remained undiagnosed until adolescence and resulted in short stature and intellectual impairment because of untreated mild hypothyroidism throughout the neonatal period and childhood.

Most of the positive cases seem to result from iodine deficiency, which is more prevalent in the mountainous regions of Italy due to poor education on the use of salt iodate. New risk values are being evaluated based on spot testing on the third day of life since the Health Service legislation has reduced hospitalization periods. FT4 serum levels, as well as TSH and T4, measured in control cases allow for better detection of cases of hypothalamic and pituitary hypothyroidism (with FT4 decrease).

## CONCLUSION

The ongoing study of new cut-off values in relation to date of birth and the introduction of FT4 control serum levels seems to prevent repeated checks and promote quicker intervention by the physicians. This prevents difficult investigations of heterodimer partner, the retinoid X receptor γ (RXRγ), iodothyronine deiodinase type 2 enzyme (D2) in the pituitary and hypothalamus. A mutation of the thyroid-hormone-receptor, D2 or RXRγ seems to be the key to the new phenotype PTFH, as previously examined by other researchers (Frankton *et al*, 2000).

## REFERENCES


Frankton S, Karmali R, Mirkinen, Bergmann P, Fuss M, Williams GR. Pituitary-thyroid feedback hypersensitivity as a novel cause of hypothyroidism in children. *Lancet* 2000;356:1238-40.

Hubl W, Meisnner D, Demant T, Becker W, Hormann R, Bach M, Mack M. Evaluation of the LIAISON thyroid chemoluminescence immunoassays. *Clin Lab* 2000;46:181-9.

Surks MI, Ocampo E. Subclinical thyroid disease. *Am J Med* 1996;100:217-23.