

## Thyroid hormone-free albumin: Charcoal treatment or resin treatment

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Free thyroid hormone measurement by means of immunoassay kits is greatly influenced by the altered serum albumin and free fatty acid (FFA) levels. In the evaluation of these kits, it is therefore essential to study the interferences due to these factors by adding FFA or thyroid hormone-free human serum albumin (HSA) to the assay mixture, but little attention has been paid to the selection of albumin.

In the present study, FFA content in various preparations of thyroid hormone-free HSA was compared. Charcoal-treated HSA was free from both thyroid hormone and FFA, whereas anion exchange resin-treated HSA was only free from thyroid hormone. Commercially available "FFA-free HSA" was also free from thyroid hormone. Our results suggest that attention must be paid to the nature of albumin when studying the interference by albumin in free thyroid hormone measurement and that commercially available "FFA-free HSA" is a ready-to-use thyroid hormone-free HSA when HSA free from both FFA and thyroid hormone is desired.

**Key words:** free thyroid hormone, charcoal, resin, albumin

### INTRODUCTION

FREE THYROID HORMONE measurement has been used in routine clinical work, since the development of the immunoassay kit. These kits, however, have been reported to be interfered with by the alteration of serum components, especially free fatty acid (FFA) and albumin.<sup>1,2</sup> In the evaluation of possible interferences by these factors, the addition of FFA or thyroid hormone-free HSA to the incubation mixture has often been employed, but the FFA content in HSA preparations has received little attention, despite the well-known property of albumin to bind FFA. We have therefore studied the FFA content in various preparations of thyroid hormone-free HSA.

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### MATERIALS AND METHODS

The following HSAs were purchased from Sigma (St. Louis, MO, USA): i.e. HSA fraction V (catalog number A-1653) and FFA-free HSA (catalog number A-3782). Charcoal (Norit A) was obtained from Nacarai Tesque (Kyoto). Anion exchange resin (AG-1X-8, chloride form) was from Biorad (Hercules, CA, USA).

Charcoal-treated thyroid hormone-free HSA (C-HSA) was prepared by treating HSA fraction V with charcoal, followed by ultracentrifugation and Millipore filtration.<sup>3</sup> Resin-treated thyroid hormone-free HSA (R-HSA) was also prepared.<sup>4</sup> In brief, HSA solution was treated with anion exchange resin (AG-1X-8) twice, followed by resin removal by ultracentrifugation and Millipore filtration.

The concentrations of T<sub>4</sub> and T<sub>3</sub> were assayed with commercial kits for total T<sub>4</sub> (SPAC T<sub>4</sub> RIA kit) and total T<sub>3</sub> (T<sub>3</sub> RIA BEADS kit). The lowest detectable concentrations were 1 µg/dl for T<sub>4</sub>, and 25 ng/dl for T<sub>3</sub>.

**Table 1** Thyroid hormone concentration in various HSA preparations

	Concentration	T <sub>4</sub> (μg/dl)	T <sub>3</sub> (ng/dl)
Sigma A-1653 (fraction V)	10%	4.6	65
Charcoal-treated A-1653	10%	u.d.	u.d.
	20%	u.d.	u.d.
Resin-treated A-1653	10%	u.d.	u.d.
	20%	u.d.	u.d.
Sigma A-3782 ("FFA-free")	10%	u.d.	u.d.
	20%	u.d.	u.d.

Data are mean of duplicate determinations. Each HSA preparations were dissolved in phosphate buffered saline as 10 or 20% solution. The abbreviations are as follows, FFA: free fatty acid, u.d.: undetectable.

**Table 2** Free fatty acid (FFA) concentration in various HSA preparations

	Concentration	FFA
Sigma A-1653 (fraction V)	10%	1.99
Charcoal-treated A-1653	10%	0.05
Resin-treated A-1653	10%	1.76
Sigma A-3782 ("FFA-free")	10%	0.02

Data are mean of duplicate determinations. Each HSA preparations were dissolved in phosphate buffered saline as 10% solution. FFA is the abbreviation for free fatty acid.

## RESULTS

Thyroid hormone concentrations in the various albumin preparations were compared (Table 1). The 10% solution of untreated HSA (fraction V) contained a significant amount of thyroid hormone. In contrast, thyroid hormone concentrations in 10% solutions of C-HSA and R-HSA were below the detectability limits (< 1.0 μg/dl for T<sub>4</sub> and < 25 ng/dl for T<sub>3</sub>). Even the 20% solution of C-HSA and R-HSA did not contain a measurable amount of T<sub>4</sub> or T<sub>3</sub>. Commercially available "FFA-free HSA" was also free from T<sub>4</sub> and T<sub>3</sub>.

In contrast, FFA content in C-HSA differed greatly from that in R-HSA (Table 2). FFA was almost completely removed from C-HSA, but it was hardly affected in R-HSA.

## DISCUSSION

Free thyroid hormone reflects the biologically active component of the circulating thyroid hormone, and its concentration is one of the most reliable parameters of thyroid hormone status. Traditionally, equilibrium dialysis and ultrafiltration have been the standard methods for free thyroid hormone determination, but these assays are cumbersome, time-consuming, and were not routinely employed.<sup>1</sup>

With the development of analog-based radioimmunoassay kits, free thyroid hormone has come to be commonly used in clinical work. In this method, radiolabeled thyroid hormone analog, which binds to thyroid hormone antibodies, but not to thyroxine binding protein (TBG), was employed, but the analog-based measurements were shown to be affected by the altered serum albumin and FFA concentrations, and the presence of autoantibodies to thyroid hormone.<sup>1</sup> Newer kits, such as Amerlex MAB (Kodak Diagnostics)<sup>5</sup> and Model FT4 (Medipysics)<sup>6</sup> were therefore developed to minimize interference by albumin and FFA.

In evaluating the validity of free thyroid hormone assay kits, it is therefore crucial to study possible interference by FFA and albumin. Of the fatty acids, oleic acid is considered to be the major FFA likely to affect the assay and has been used as a standard FFA,<sup>7</sup> but little attention has been paid to the selection of albumin. Hashimoto et al. reported that the various lots of commercially available albumin exert different effects when added to the Amerlex free T<sub>4</sub> assay system.<sup>3</sup> They also reported that charcoal-treated T<sub>4</sub>-free human serum albumin (HSA) had far less effect on the Amerlex free T<sub>4</sub> assay. Thus they concluded that HSA-bound T<sub>4</sub> rather than HSA itself interferes with the assay and stressed the importance of using thyroid hormone-free HSA.<sup>3</sup> As far as we know, their paper was the first to call attention to the nature of the albumin, but they did not consider using the method to prepare thyroid hormone-free HSA.

The present paper has clearly demonstrated that charcoal treatment removes both FFA and thyroid hormone, and resin treatment selectively removes thyroid hormone without affecting FFA. These results are conceivable considering the fact that charcoal is a rather nonspecific absorber and that the anion exchange resin (AG-1X-8) has a high affinity for phenolate ion. Because of its outer ring phenolate structure, thyroid hormone is selectively removed.<sup>4</sup> Since FFA and albumin are the two major serum components which interfere with the free thyroid hormone assay, attention must be paid on the nature of the albumin employed when studying interference by albumin in the free thyroid hormone assay.

Since the commercially available FFA-free albumin is usually prepared by charcoal treatment, it seemed likely that commercially available "FFA-free HSA" is also a thyroid hormone-free HSA. Indeed, as shown in Tables 1 and 2, an albumin preparation sold as "FFA-free HSA" was free from both FFA and thyroid hormone. Thus our data also show that commercially available "FFA-free HSA" can be used as a ready-to-use thyroid hormone-free HSA, when HSA free from both FFA and thyroid hormones is desired.

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