Abstract.—Objective. To analyse the behaviour of serum thyroglobulin (Tg), antithyroglobulin antibodies (TgAb), thyrotropin (TSH), free thyroxine (FT4) and total triiodothyronine (TT3) levels at each time during the rhTSH stimulation protocol in patients with differentiated thyroid carcinoma (DTC).

Materials and methods. We carried out 117 rhTSH stimulations in DTC patients. We determined the serum levels of Tg, TgAb, TSH, FT4 and TT3 at baseline and 24, 48 and 96 hours after beginning stimulation, using RIA or IRMA. The software program SPSS 15.0 was used for statistical analysis of data.

Results. We found statistically significant differences between the mean Tg values at different times (2.08 ng/ml baseline; 2.64 ng/ml at 24 hours; 4.98 ng/ml at 48 hours; 6.59 ng/ml at 96 hours), reaching maximum values at 96 hours. During this time, we observed the highest percentage of pathological values. After administration of rhTSH, there was a significant increase in the mean TSH value (98.88 mIU/l at 24 hours; 111.10 mIU/l at 48 hours). The mean TSH value at 96 hours decreased approximately 5 times with respect to the mean 48 hour value. We did not observe changes in the TgAb, FT4 or TT3 levels.

Conclusions. The assessment of Tg after rhTSH stimulation should be performed 96 hours after beginning stimulation. Administration of rhTSH causes a significant elevation in serum TSH levels, without modifying serum TgAb, FT4 or TT3 levels.

KEY WORDS: differentiated thyroid carcinoma, recombinant human TSH, thyroglobulin, antithyroglobulin antibodies, thyrotropin, thyroxine, triiodothyronine.

INTRODUCTION
The treatment for most patients with differentiated thyroid cancer is total or almost total thyroidectomy, followed by an ablative dose of 131I. Patients are then...
treated with synthetic thyroid hormones, mainly sodium thyroxine (T4). The synthetic T4 hormone substitutes the endogenous hormones and suppresses the secretion of thyrotropin (TSH), minimising the tumour growth induced by the latter. Low TSH levels also reduce the uptake of iodine and the secretion of thyroglobulin (Tg) by the tumour or residual thyroid tissue. Therefore, for the 131I diagnostic/therapeutic procedure, thyroid hormone therapy must be withdrawn, elevating the endogenous serum TSH levels and increasing the sensitivity of the serum Tg test. During the withdrawal of synthetic thyroid hormones, patients may experience a period of severe hypothyroidism. Recombinant human TSH (rhTSH) today constitutes an alternative to hormone deprivation in the management of differentiated thyroid carcinoma (DTC). It is a heterodimeric glycoprotein produced by recombinant DNA technology, with an amino acid sequence identical to that of human pituitary TSH. Its binding to TSH receptors in healthy epithelial cells or in well-differentiated thyroid carcinoma stimulates the uptake and organification of iodine, as well as the synthesis and secretion of Tg, triiodothyronine and thyroxine. This allows patients to continue hormone suppression therapy, avoiding the signs and symptoms of hypothyroidism.

The rhTSH stimulation protocol consists of the intramuscular administration of 0.9 mg of rhTSH on two consecutive days. Twenty-four hours after administration of the second dose of rhTSH, 131I is administered orally, and 48 hours after the administration of sodium iodide, a whole body scan and determination of serum Tg are performed. In addition to its use for diagnostic purposes, rhTSH is indicated for pre-therapeutic stimulation in low risk post-thyroidectomy patients maintained on hormone suppression treatment, for the ablation of residual thyroid tissue with 3.7 GBq of 131I.

The pharmacokinetic profile of rhTSH has been established in various studies. Pellegriti et al. found great variability in the time to obtaining the maximum serum Tg value after administration of rhTSH. Recent guidelines and various authors recommend the determination of this tumour marker 96 hours after beginning stimulation with rhTSH. Other authors, for various reasons, perform the aforementioned measurement at 48 hours, coinciding with the administration of 131I.

There are practically no studies on the serum levels of thyroid hormones after administration of rhTSH in DTC patients. Only David et al. did not find variations in the free thyroxine or free triiodothyronine levels.

The objective of our study was to analyse the behaviour of serum Tg, antithyroglobulin antibody (TgAb), TSH, free thyroxine (FT4) and total triiodothyronine (TT3) levels at each time in the rhTSH stimulation protocol, in DTC patients.

**MATERIALS AND METHODS**

We conducted a descriptive study, in which we included all DTC patients in follow-up, after rhTSH stimulation in the Hospital Universitario Reina Sofía (Córdoba) Nuclear Medicine Clinical Management Unit, from January 2003 to January 2005.

Using radioimmunoassay (RIA) or radioimmunometric assay (IRMA), we determined the serum levels of Tg, TgAb, TSH, FT4 and TT3 at each time in the stimulation protocol, i.e. we determined the baseline levels (first intramuscular dose, 0.9 mg rhTSH) as well as the levels 24 hours (second intramuscular dose, 0.9 mg rhTSH), 48 hours (oral administration of a 148 MBq 131I capsule) and 96 hours after beginning the stimulation (scan). All measurements were performed in duplicate.

The serum Tg and TSH concentrations were determined using IRMA and the TgAb and thyroid hormones using RIA. The TSH, FT4, TT3 and TgAb reference values were established from healthy donor sera. In the case of Tg, we took the value recommended by current guidelines (< 1 ng/ml) as the reference limit. Table 1 shows the commercial kit used in the determination and the reference range.

We used the “Stratec RS300” and “Packard COBRA II 5005” solid scintillation gamma counters (NaI(Tl), multi-well).

Statistical analysis of the data was performed using the SPSS program, version 15.0. For the analysis of qualitative variables, the absolute and relative frequencies were determined, and the minimum, maximum, mean and standard deviation were calculated for the quantitative variables.

The different groups were compared by applying the Chi-squared test, Student’s t-test or the corresponding non-parametric test. The test was considered statistically significant when the p value was less than 0.05.
RESULTS

A total of 117 rhTSH stimulations were carried out. Table 2 and figure 1 show the behaviour of Tg after rhTSH administration. An increase in the mean serum Tg levels with respect to the mean baseline value can be observed (2.08 ng/ml basal; 2.64 ng/ml at 24 hours; 4.98 ng/ml at 48 hours and 6.59 ng/ml at 96 hours). The differences found were statistically significant. The mean Tg value reached a maximum 96 hours after beginning the stimulation.

If we categorise the Tg variable as positive (> 1 ng/ml) and negative (< 1 ng/ml), in figure 2 we can see that the highest percentage of pathological values, or positive Tg, is found at 96 hours (5.1 % basal; 9.4 % at 24 hours; 16.2 % at 48 hours and 32.2 % at 96 hours).

Table 3 shows the serum TgAb levels after administration of rhTSH alfa. When the results obtained at the different times were compared, no statistically significant differences were found between them.

After rhTSH administration, a statistically significant increase was observed in the mean serum TSH concentration (table 4 and fig. 3) at 24 hours (98.88 mIU/l) and 48 hours (111.10 mIU/l). The mean serum TSH value at 96 hours decreased approximately 5 times with respect to the mean 48 hour value.

Table 5 shows the serum FT4 levels at each time during the rhTSH stimulation protocol. In this case, no statistically significant differences were found

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**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Method of determination</th>
<th>Commercial kit</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg</td>
<td>IRMA</td>
<td>SELco® Tg (Medipan)</td>
<td>0–1 ng/ml</td>
</tr>
<tr>
<td>TgAb</td>
<td>RIA</td>
<td>SELco® anti-Tg (Medipan)</td>
<td>0–100 IU/ml</td>
</tr>
<tr>
<td>TSH</td>
<td>IRMA</td>
<td>SELco® TSH rapid (Medipan)</td>
<td>0.3–3.5 mIU/l</td>
</tr>
<tr>
<td>FT4</td>
<td>RIA</td>
<td>RIA-ghost® FT4 (Cis bio international)</td>
<td>0.7–1.8 ng/dl</td>
</tr>
<tr>
<td>T3T</td>
<td>RIA</td>
<td>T3-CTK (DiaSorin)</td>
<td>80–200 ng/dl</td>
</tr>
</tbody>
</table>

TgAb: serum antithyroglobulin antibodies; FT4: serum free thyroxine; IRMA: radioimmunometric assay; RIA: radioimmunoassay; Tg: serum thyroglobulin; TSH: serum thyrotropin; T3T: serum total triiodothyronine.

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**Table 2**

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>116</td>
<td>0.50</td>
<td>72.60</td>
<td>2.08</td>
<td>9.41</td>
</tr>
<tr>
<td>24 hours</td>
<td>117</td>
<td>0.50</td>
<td>82.00</td>
<td>2.64</td>
<td>11.90</td>
</tr>
<tr>
<td>48 hours</td>
<td>116</td>
<td>0.50</td>
<td>173.10</td>
<td>4.98</td>
<td>22.46</td>
</tr>
<tr>
<td>96 hours</td>
<td>101</td>
<td>0.50</td>
<td>229.00</td>
<td>6.59</td>
<td>29.52</td>
</tr>
</tbody>
</table>

N: number of measurements; rhTSH: recombinant human thyrotropin. Comparison of means with respect to the baseline measurement: *P = 0.035; **P = 0.030; ***P = 0.028.

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**Table 3**

| **SERUM THYROGLOBULIN (ng/ml) AFTER rhTSH STIMULATION** |
|----------------|----------------|----------------|
| Time       | N  | Minimum | Maximum | Mean   | Standard deviation |
| Baseline   | 116| 0.50    | 72.60   | 2.08   | 9.41               |
| 24 hours   | 117| 0.50    | 82.00   | 2.64   | 11.90              |
| 48 hours   | 116| 0.50    | 173.10  | 4.98   | 22.46              |
| 96 hours   | 101| 0.50    | 229.00  | 6.59   | 29.52              |

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**Fig. 1.**—Mean serum thyroglobulin (Tg) levels (ng/ml) after recombinant human thyrotropin stimulation.

**Fig. 2.**—Serum thyroglobulin (Tg) after recombinant human thyrotropin stimulation. (–): Tg negative (< 1 ng/ml); (+): Tg positive (> 1 ng/ml).
between the mean FT4 values at different times (2.34 ng/dl basal; 2.38 ng/dl at 24 hours; 2.26 ng/dl at 48 hours and 2.37 ng/dl at 96 hours).

Statistically significant differences were not found either between the mean serum TT3 values after administration of rhTSH alfa (table 6) (94.72 ng/dl basal; 87.84 ng/dl at 24 hours; 92.70 ng/dl at 48 hours and 91.63 ng/dl at 96 hours).

DISCUSSION

Administration of rhTSH causes a significant elevation in serum Tg levels. In most cases, the maximum Tg value was observed 96 hours after the first rhTSH dose. These results are similar to those obtained by Haugen et al7 in patients with DTC and by Pacini et al 10, in 72 patients with DTC and non-detectable Tg levels during T4 hormone therapy.

In our study, the Tg was positive in 45 out of 117 cases. The maximum Tg value was obtained at 24 hours in 2 cases, at 48 hours in 2 cases and at 96 hours in 38 cases. In the 3 remaining cases, the maximum value was the same 48 and 96 hours after beginning the stimulation.
Pellegriti et al. observed great variability in the time to obtaining the maximum serum Tg value after administration of rhTSH, which they partially explained as due to the variability in serum TSH levels, inversely related to the patient’s body surface. This study was conducted on 13 patients with DTC, with persistent disease and low Tg levels on T4 treatment. The authors proposed successive Tg determinations within the first week to locate the maximum value.

Jiménez-Hoyuela et al.1 in 102 DTC patients in follow-up, did not find any positive case at 48 hours and negative case at 96 hours after initial stimulation; therefore, they considered serum Tg measurement on the fifth day after beginning the procedure to be adequate.

Some authors12,13, for various reasons (infrastructure, etc.), measure the Tg on the day of 131I administration (48 hours). In our study, we found 25 cases in which the Tg was negative at that time, and became positive at 96 hours. This means 21.4 % of the total cases and 64.1% of cases in which they became positive. Therefore, to reduce the number of false negatives, we believe it is advisable to determine the level at 96 hours as well as the baseline level.

Due to the possible interference of TgAb in the Tg level, it is recommended to measure both together4,15,16. The results presented are those obtained in all the cases. If we exclude those Tg measurements for which the TgAb were positive, the results obtained are similar (mean serum Tg: 2.21 ng/ml basal; 2.77 ng/ml at 24 hours; 5.34 ng/ml at 48 hours and 7.01 at 96 hours).

We did not find variations in the TgAb levels after administration of rhTSH alfa. The TgAb were positive in 7.7 % of cases at time zero, as well as at 24 and 48 hours. This percentage was somewhat lower at 96 hours due to a higher percentage of lost values.

The behaviour of rhTSH in serum was similar to that described in previous studies 6,7,10, reaching the maximum value 48 hours after the first rhTSH dose. The serum TSH value at 24 and 48 hours was always higher than 30 mIU/l, this being the concentration required to ensure effective 131I uptake by the thyroid remnants and increased release of Tg2,4.

Castagna et al.17 studied the influence of body composition on the rhTSH peak after rhTSH administration and found an association between this peak and the lean body mass in 105 DTC patients.

The serum FT4 and TT3 values did not change at any time after administration of TSH alfa.

One of the limitations of our study is the loss of data, basically due to insufficient blood sample volume for the determination of all the study variables. In the worst case, we had 81 measurements (TT3 at 96 hours), a number which we consider adequate to respond to the planned objectives.

On the other hand, by not collecting the characteristics of the patients studied, we were unable to assess the influence of these factors in the response of the study variables after rhTSH administration.

CONCLUSIONS

In accordance with the results obtained, we conclude that, in thyroidectomised differentiated thyroid carcinoma patients: a) the assessment of Tg after rhTSH stimulation should be performed 96 hours after beginning the stimulation; b) the administration of rhTSH causes a significant elevation in serum TSH levels, and c) rhTSH administration does not modify serum TgAb, FT4 or TT3 levels.

BIBLIOGRAPHY