Background: One night of sleep deprivation induces a brief remission in about half of depressed patients. Subclinical hypothyroidism may be associated with depression, and changes in hypothalamic-pituitary-thyroid function may affect the mood response to sleep deprivation. We wished to define precisely the status of the hypothalamic-pituitary-thyroid axis of depressed patients during sleep deprivation and the possible relationship of hypothalamic-pituitary-thyroid function to the mood response.

Methods: We studied 18 patients with major depressive disorder and 10 normal volunteers. We assessed mood before and after sleep. We measured serum thyrotropin every 15 minutes during the night of sleep deprivation, thyrotropin bioactivity, the thyrotropin response to protirelin the next afternoon, and other indexes of hypothalamic-pituitary-thyroid function. To determine if the changes were limited to the hypothalamic-pituitary-thyroid axis, we measured serum cortisol, which also has a circadian secretory pattern.

Results: Nocturnal serum thyrotropin concentrations were consistently higher in responders, entirely because of elevated levels in the women responders. Responders had exaggerated responses to protirelin the next afternoon. The bioactivity of thyrotropin in non-responders was significantly greater than in responders (F_{1,8.99} = 7.52; P = .02). Other thyroid indexes and serum cortisol concentrations were similar among groups.

Conclusions: Depressed patients have mild compensated thyroid resistance to thyrotropin action, not subclinical autoimmune primary hypothyroidism. Sleep deprivation responders compensate by secreting more thyrotropin with normal bioactivity; non-responders compensate by secreting thyrotropin with increased bioactivity.

Arch Gen Psychiatry. 2001;58:77-83
SUBJECTS AND METHODS

SUBJECTS

The study protocol was approved by the Vanderbilt University Institutional Review Board—Human Subjects (Nashville, Tenn.), and written informed consent was obtained from all subjects. We studied 8 male and 10 female outpatients aged 28 to 62 years (mean±1 SD, 43.6±9.31 years), diagnosed as having MDD according to the Structured Clinical Interview for DSM-III-R—Patient Version,30 who scored at least 18 on the first 17 items of the Hamilton Rating Scale for Depression.31 Patients had no history of substance abuse for 6 months and no lifetime history of psychosis or bipolar disorder. Before SD, none had taken lithium carbonate for 3 months; fluoxetine, benzodiazepines, or other psychotropics with long half-lives for 3 weeks; or other antidepressants for 2 weeks. We recruited some patients from those being seen for outpatient treatment, and other patients and all normal volunteers from advertisements posted in the community. We studied 10 normal volunteer controls of similar age and sex (5 men and 5 women aged 29 to 50 years [mean±SD, 41.0±7.44 years]). Controls were free of Axis I mental disorders according to the Structured Clinical Interview for DSM-III-R—Patient Version and scored less than 8 on the Hamilton Rating Scale for Depression.31 All participants were physically healthy as determined by clinical history, physical examination, routine serum chemistry studies, and electrocardiogram and were able to give informed consent. None had a personal or family history of thyroid disease or evidence of thyroid dysfunction. We excluded any subject whose urine tested positive for illicit drugs and any woman who was pregnant, lactating, or of childbearing potential and not using contraceptive methods.

PROCEDURES

Participants were admitted to the Vanderbilt General Clinical Research Center during the morning of the first day. Meal times were 8 AM, noon, and 8 PM. Participants remained awake and ambulated and signed a record sheet every 30 minutes for 36 hours under constant observation. We assessed severity of depression at recruitment, the day before SD, and 1 week after SD by means of the Sleep Deprivation Depression Rating Scale (SDDRS), a modified Hamilton Rating Scale for Depression.32 This scale excludes insomnia, weight loss, diurnal variation, depersonalization, paranoia, and obsessive-compulsive items and adds elements that rate fatigue, social withdrawal, increased appetite, increased eating, carbohydrate craving, weight gain, and hypersomnia. We defined response to SD as 30% or more reduction in the SDDRS score the morning after SD.25,32 Two years later, we recalled the 14 available patients with MDD and all 10 controls for interval history; Structured Clinical Interview for DSM-III-R—Patient Version (patients with MDD), physical examination, and thyroid function studies.

HORMONE ASSAYS

We measured serum thyrotropin with an immunoradiometric assay (Allegro; Quest, San Juan Capistrano, Calif), and thyrotropin-α and thyrotropin-β subunits with in-house radioimmunoassays with sensitivities of 5 and 2.8 pmol/L of plasma, respectively. Thyrotropin cross-reacted 27% and 3.1% in the thyrotropin-α and thyrotropin-β radioimmunoassays, respectively; thyrotropin-α cross-reacted 0.6% and 1.2% in the thyrotropin immunoradiometric assay and thyrotropin-β radioimmunoassay, respectively; and thyrotropin-β cross-reacted less than 0.0004% and less than 0.01% in the thyrotropin immunoradiometric assay and thyrotropin-α radioimmunoassay, respectively. We measured thyrotropin bioactivity in pooled aliquots of all nocturnal samples after immunofinity purification (recovery, 48% to 68%) and ultrafiltration.33-35 Results (mean±1 SD of 3 experiments) are expressed as the bioactivity to immunoreactivity ratio (B/I). We measured serum free thyroxine, total triiodothyronine, anti–thyroid peroxidase and anti–thyroglobulin antibody titers, and serum cortisol by means of commercial kits.

RESULTS

MOOD EFFECTS OF SLEEP DEPRIVATION

The mean SDDRS score of all 18 patients with MDD declined by 34% (range, −7% to 78%), from 20.7±5.2 (mean±1 SD) to 13.1±5.3; that of the 10 responders, by 56% (range, 38% to 78%), from 22.2±5.5 to 9.8±4.0; and that of the 8 nonresponders, by 6% (range, −7% to 27%), from 18.8±4.5 to 17.3±4.2. The ANOVAs disclosed no differences in baseline scores between responders and nonresponders (F1,16 =2.07; P=.17), but a decline for the whole MDD group (F1,16 =69.96;
STATISTICAL ANALYSES

The primary analyses compared the nocturnal serum thyrotropin levels and thyrotropin B/I during SD and serum thyrotropin responses to protirelin (peak level minus the mean of 2 prechallenge baseline levels) of responders, nonresponders, and normal control subjects. We computed nocturnal serum thyrotropin means in 4-hour sampling blocks (10 PM to 2 AM, 2:15 AM to 6 AM, and 6:15 AM to 10 AM).

Levene tests indicated significant between-group heterogeneity of variance in thyrotropin B/I and protirelin response ($F_{1,24}=8.05; P<.005$; and $F_{1,24}=4.27; P<.05$, respectively). A likelihood ratio test indicated significant between-group heterogeneity of across-time covariance matrices in nocturnal serum thyrotropin ($\chi^2_{12}=35.87; P<.001$). One- and 2-way analyses of variance (ANOVAs) often demonstrate excessive type 1 error rates and/or insufficient power with heterogeneous variances and unequal sample sizes. Consequently, we conducted generalized Welch approximate degrees of freedom (WADF) tests to assess effects in our 3 primary measures. The WADF tests do not assume equality of population variances across groups, and their type 1 error performance and power are typically superior to those of ANOVA under variance heterogeneity.

We conducted a group (responder, nonresponder, control) × time (blocks 1-3) WADF test on nocturnal serum thyrotropin levels. Our original intent was to assess only group effects, but inspection of cell means indicated potential main effects or interactions involving sex. Therefore, we also conducted group × sex × time WADF tests to assess effects in our 3 primary measures. The WADF tests do not assume equality of population variances across groups, and their type 1 error performance and power are typically superior to those of ANOVA under variance heterogeneity.

We also computed 2-tailed WADF planned comparisons (α=.05) that compared responders and nonresponders on the 3 primary thyrotropin measures. We conducted post hoc WADF tests of the pairwise differences between the control group and the other 2 groups only when omnibus WADF analyses yielded significant main effects for group. Post hoc comparisons followed the Fisher least significant difference strategy, which is optimal when the group number equals 3 and only pairwise post hoc comparisons are contemplated. Significant group × sex interactions were followed by WADF omnibus ANOVAs that compared the 3 groups within each category of sex. We set the critical α level of these analyses at .05/2=.025, where 2 is the number of sexes, to control familywise type 1 error rates. Follow-up contrasts used the same critical α level.

In addition to between-group analyses, we computed Spearman correlations to assess the relationship between pre- and post-SD SDDRS scores of the depressed patients and each of our 3 primary thyrotropin measures.

We conducted ANOVAs (or WADF tests, when variance heterogeneity was observed) comparing responders, nonresponders, and controls on 3 additional thyrotropin measures during SD (thyrotropin-α, thyrotropin-β, and the ratio of thyrotropin-β to thyrotropin-α [thyrotropin-β/thyrotropin-α]). For brevity, we report only the group × sex analyses. We set the critical α level for each effect to .05/3=.0167, where 3 is the number of thyrotropin measures. We also tested for between-group differences in thyrotropin measures at follow-up. One-way between-group tests were performed because data from only 4 nonresponders were available. We conducted a group × sex × time ANOVA on nocturnal serum cortisol during SD. As was true of the primary dependent measures, resampling analyses (bootstrapping and/or permutation tests) of each of these supplementary measures produced results and conclusions that were identical to those of the normal-theory WADF tests and ANOVAs. For brevity, we present only the latter results.

Eight of the 10 responders had 1 or more nocturnal thyrotropin values greater than normal (>4.2 mU/L), as did 1 of 8 nonresponders and 2 of 10 controls. No participant had a concentration less than normal (<0.9 mU/L). The planned contrast indicated higher thyrotropin levels in responders than nonresponders ($F_{1,15.73}=7.20; P=.02$). Omnibus group × time WADF analysis of the 4-hour pools indicated a main effect for group ($F_{2,13.07}=6.64; P=.009$). Post hoc contrasts indicated that responders had higher levels than controls ($F_{1,13.52}=13.53; P<.001$). A time effect ($F_{2,13.07}=13.23; P<.001$), but no group × time interaction ($F_{2,13.07}=0.82; P=.53$), was observed. Thyrotropin level peaked during
the period from 2:15 to 6 AM (post hoc contrast \( F_{1,19.70} = 25.90; P < .001 \)).

The group \( \times \) sex \( \times \) time unweighted means WADF analysis also indicated a main effect for group (\( F_{2,10.76} = 6.72; P = .01 \)) and an identical pattern of between-group differences on planned and post hoc contrasts (responders vs nonresponders, \( F_{1,9.23} = 6.50; P = .03 \); responders vs controls, \( F_{1,10.97} = 12.75; P = .004 \)). However, the effects of group were moderated by sex (group \( \times \) sex interaction, \( F_{2,6.34} = 6.16; P = .008 \)). Follow-up WADF analyses indicated between-group differences for women (\( F_{2,6.34} = 18.68; P = .002 \)), but not for men (\( F_{2,6.00} = 0.05; P = .95 \)). Female responders had higher thyrotropin levels (\( 6.45 \pm 1.09 \) mU/L) than female nonresponders (\( 3.06 \pm 0.80 \) mU/L) (\( F_{3,30} = 30.02; P = .002 \)) and female controls (\( 2.80 \pm 1.19 \) mU/L) (\( F_{1,8.22} = 27.95; P < .001 \)).

**NOCTURNAL SERUM THYROTROPIN B/I RATIOS**

Planned WADF contrasts indicated that nonresponders had higher ratios (\( 2.92 \pm 1.36 \)) than responders (\( 1.50 \pm 0.58 \)) (1-way design contrast, \( F_{1,8.99} = 7.52; P = .02 \); 2-way design contrast, \( F_{1,8.60} = 6.59; P = .03 \)). Omnibus tests suggested a trend toward overall between-group differences (1-way design group, \( F_{2,11.76} = 3.63; P = .054 \); 2-way design group, \( F_{2,13.71} = 3.15; P = .01 \)) (control mean, \( 1.74 \pm 0.45 \)). No main effects or interactions involving sex were found in the 2-way analyses (sex main effect, \( F_{1,10.16} = 0.006; P = .94 \); group \( \times \) sex interaction, \( F_{2,7.37} = 2.18; P = .18 \)).

**RESPONSE TO PROTIRELIN STIMULATION**

All groups responded to protirelin (\( F_{1,11.23} = 74.45; P < .001 \)) ([Figure 2](#)). Eight of the 10 responders had higher than normal thyrotropin responses (\( > 28.3 \) mU/L for women, >23.8 mU/L for men), as did 2 of the 6 nonresponders and 4 of the 10 controls; no participant had a less than normal response (\(< 10.1 \) mU/L for women, \(< 3.8 \) mU/L for men). Although 1-way omnibus WADF analysis indicated only a trend for group (\( F_{2,11.85} = 3.41; P = .06 \)), the planned contrast indicated that responders had greater thyrotropin increments than nonresponders (\( F_{1,10.20} = 7.08; P = .02 \)). The planned contrast linked to the group \( \times \) sex WADF analysis also indicated that responders had greater thyrotropin increments than nonresponders (\( F_{1,8.80} = 8.49; P = .02 \)). In addition, the omnibus group \( \times \) sex WADF analysis indicated between-group differences in thyrotropin increments (group main effect, \( F_{2,12.20} = 4.04; P = .04 \)). Follow-up contrasts of the omnibus effect indicated that responders had greater increments than controls (\( F_{1,9.92} = 7.42; P = .02 \)). Nonresponders and controls did not differ (\( F_{1,10.77} = 0.06; P = .82 \)). Women responded more robustly than men (\( F_{1,10.80} = 9.49; P = .01 \)). The group \( \times \) sex interaction was not significant (\( F_{2,12.20} = 2.60; P = .11 \)).

**EFFECT SIZES**

We computed Cohen’s effect size index \( d^{2} \) to estimate the magnitude of differences between the responders and nonresponders for the 3 thyrotropin measures. The \( d \) values for nocturnal thyrotropin, thyrotropin B/I ratios, and thyrotropin increments in response to challenge were 1.26, 1.45, and 1.07, respectively. In light of Cohen’s stated criteria of 0.5 for a medium effect size and 0.8 for a large effect size, these \( d \) values underscore the magnitude of the thyrotropin differences between responders and nonresponders.

**CORRELATIONS BETWEEN PRIMARY THYROTROPIN MEASURES AND SDDRS SCORES**

We computed Spearman correlations among pre- and post-SD SDDRS scores and the 3 primary thyrotropin measures ([Table 1](#)). Post-SD SDDRS scores were significantly correlated with all 3 thyrotropin measures despite the relatively small sample sizes: lower post-SD SDDRS scores were associated with higher nocturnal se-
Levene tests indicated variance heterogeneity in thyrotropin-α (F2,23 = 12.04; P < .001). The WADF and resampling analyses of thyrotropin-α indicated no significant effects involving group (main effect, F2,11.24 = 0.98; P = .40; group × sex interaction, F2,11.24 = 0.37; P = .70) (Table 2). The ANOVAs and resampling analyses of thyrotropin-β and thyrotropin-β/thyrotropin-α indicated no significant effects involving group (thyrotropin-β group main effect, F2,22 = 0.28; P = .76; thyrotropin-β group × sex interaction, F2,22 = 0.48; P = .62; thyrotropin-β/thyrotropin-α group main effect, F2,22 = 0.71; P = .50; thyrotropin-β/thyrotropin-α group × sex interaction, F2,22 = 1.18; P = .33).

TWO-YEAR FOLLOW-UP EVALUATION

Table 2. Immunoreactive Thyrotropin Subunit Measures During Sleep Deprivation

<table>
<thead>
<tr>
<th>Thyrotropin-α, pmol/L</th>
<th>Thyrotropin-β, pmol/L</th>
<th>Thyrotropin-β/thyrotropin-α, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 10)</td>
<td>Normal Control Subjects (n = 10)</td>
<td></td>
</tr>
<tr>
<td>9.37 ± 1.19</td>
<td>101.0 ± 43.5</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>8.55 ± 2.26</td>
<td>86.3 ± 63.9</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>8.62 ± 1.39</td>
<td>140.0 ± 127.0</td>
<td>0.10 ± 0.06</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.

Mean nocturnal serum cortisol concentrations demonstrated a normal circadian increase in all groups (time, F2,42 = 39.72; P < .001). Cortisol levels were similar and normal in all groups (responders, 256 ± 68 nmol/L; nonresponders, 281 ± 70 nmol/L; controls, 238 ± 39 nmol/L [group main effect, F2,21 = 1.65; P = .22]).

Consistent with previous reports, 56% of our patients with MDD responded to 1 night of SD. Serum thyrotropin level increased during SD in all 3 groups, reaching a peak between 2:15 AM and 6 AM, confirming previous reports. The elevated basal serum thyrotropin level in our SD responders increased in parallel with that of nonresponders and controls during SD but was higher throughout the sampling period; mean nocturnal serum thyrotropin level was above the upper normal limit in responders, but in none of the nonresponders or controls. The correlation of increased serum thyrotropin level with clinical response was robust in our patients, consistent with some but not all studies. The changes were limited to the HPT in our patients, since the circulating concentrations of cortisol, which also has a circadian secretory pattern, were normal and similar among the 3 groups. The results suggest that altered HPT function either plays a role in MDD and the mood response to SD or is an epiphenomenon reflecting altered HPT function in both.
The increased serum thyrotropin levels, normal thyroid hormone concentrations, and exaggerated thyrotropin responses to protirelin stimulation in the SD responders are consistent with subclinical primary hypothyroidism. This autoimmune disorder occurs in about 5% of mostly postmenopausal women. Our patients had no evidence of autoimmune thyroiditis. Although the nocturnal serum thyrotropin level of SD nonresponders was normal and they did not have an exaggerated response to protirelin, their circulating thyrotropin had increased bioactivity. Furthermore, 2 years after SD, no patient with MDD was hypothyroid. If they had transient hypothyroidism caused by thyroiditis, it was of a kind not previously described. Transient thyroiditis with hypothyroidism is rare except postpartum and is usually preceded by thyrotoxicosis. However, the combination of increased serum thyrotropin level and normal serum thyroid hormone levels may explain the proposed association between MDD and subclinical primary hypothyroidism.

Our results do not indicate central (ie, hypothalamic) hypothyroidism, which is typified by high serum thyrotropin concentrations, low serum thyroid hormone levels, an exaggerated response to protirelin, low thyrotropin-B/I, and high serum concentrations of free thyrotropin-β subunit. Our responders’ thyrotropin-α and thyrotropin-β subunit concentrations and thyrotropin-β/thyrotropin-α ratios were not different from those of nonresponders or controls. Moreover, our results are not consistent with resistance to thyroid hormone action involving the pituitary gland, peripheral tissues, or both.

We were surprised to find that responders had normal and nonresponders had increased thyrotropin-B/I ratios; we anticipated decreased and normal ratios, respectively. Increased thyrotropin bioactivity, caused by decreased terminal sialic acid residues on thyrotropin carbohydrate side chains, occurs in healthy third-trimester human fetuses and patients with thyroid hormone resistance. Decreased bioactivity caused by increased sialylation is found at night in healthy subjects and during daytime in hypothyroid patients; the latter is reversed with long-term thyroxine administration. Our finding of increased thyrotropin bioactivity without evidence of thyroid hormone resistance in an adult is unprecedented.

Sleep deprivation responders had exaggerated serum thyrotropin responses to protirelin, and nonresponders had normal responses but greater thyrotropin bioactivity; none had a blunted response. Previous studies, in which protirelin usually was administered at 9 AM, report that about 30% of depressed patients have blunted thyrotropin responses. Some studies report higher afternoon than morning responses to protirelin in patients with MDD. Thus, our results may reflect either the timing of the protirelin injection or an effect of SD.

Our results suggest 2 different HPT phenomena, one associated with MDD and the other with the mood response to SD. The thyroid gland of patients with MDD appears to be resistant to thyrotropin action. This represents a novel form of transient compensated primary hypothyroidism with an unknown, but presumably central, mechanism. Although the HPT of patients with MDD appears to respond appropriately by increasing the level of biologically active thyrotropin, SD responders and nonresponders accomplish this by different means. Sleep deprivation responders appear to increase their serum thyrotropin concentration by increasing secretion of thyrotropin with normal bioactivity. In contrast, SD nonresponders appear to increase the bioactivity of their thyrotropin without increasing their thyrotropin secretion or serum thyrotropin concentration. The mechanism producing this phenomenon is also unknown. Perhaps a difference in the secretion or action of a protirelin antagonist mediates the different pituitary thyrotropin responses in the 2 groups.

The fact that increased nocturnal serum thyrotropin concentrations were limited to female patients with MDD is provocative. However, the small sizes of the groups of men and women mandate that any conclusions about sex differences be considered tentative.

The nature of the thyroid gland resistance to thyrotropin, the mechanisms that determine why and how individual patients with MDD increase either their thyrotropin secretion rate or their thyrotropin bioactivity to compensate for this resistance, and the relationship of these 2 mechanisms to MDD and SD response or nonresponse remain to be determined.


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