Serum Interleukin-6 and Thyroid Hormones in Rheumatoid Arthritis

M.L. Wellby, J.A. Kennedy, K. Pile, B.S. True, and P. Barreau

Using rheumatoid arthritis (RA) as a model, we have investigated whether the activation of the cytokine system, in particular, activation of interleukin (IL)-6 production, is a major cause of the depressed serum T_3 seen frequently in the nonthyroidal illness syndrome (NTIS). RA was chosen because it is a chronic autoimmune disease leading to increased serum IL-6 concentrations. We studied 16 untreated RA and 35 treated RA patients. Twenty-seven treated and 27 untreated patients with noninflammatory musculoskeletal symptoms served as controls. The patient groups displayed similar age distribution and nutritional status. Untreated RA patients displayed elevations of serum IL-6 (mean, 37.5 pg/mL) and C-reactive protein (CRP; mean, 41.3 mg/L), consistent with the inflammatory nature of their disease. Treated RA patients had significantly reduced serum IL-6 (mean, 9.9 pg/mL) and CRP (mean, 13.3 mg/L) compared with untreated RA patients, while untreated and treated patients with noninflammatory musculoskeletal symptoms had near normal serum IL-6 (mean, 2.5, 6.6 pg/mL, respectively) and CRP levels (mean, 5.8, 8.1 mg/L, respectively). However, there were no significant differences in serum concentrations of free T_3 (FT_3) and free T_4 (FT_4) between groups, and thyroid indices were in the normal range in RA patients. Moreover, no significant correlations between serum concentration of IL-6 and any of the thyroid hormones were demonstrated for any of the patient groups. In conclusion, we have been unable to confirm in RA that IL-6 activation leads to the low T_3 state of NTIS.

PATIENTS WITH VARIOUS acute or chronic diseases or after surgery frequently have abnormal thyroid hormone tests in the absence of symptoms and signs suggesting abnormal thyroid function. The thyroid tests are commonly characterized by subnormal serum T_3, normal serum T_4, and near normal and slightly variable serum thyroid-stimulating hormone (TSH) levels, which are inappropriately low in relationship to the low T_3 levels for the euthyroid state. This state has recently been designated as the nonthyroidal illness syndrome (NTIS).[1] The subnormal serum T_3 is a consistent hallmark of NTIS that apparently largely arises from its depressed peripheral production from T_4, secondary to reduced hepatic type 1 deiodinase (5'DI) activity.

Many acute and chronic diseases are associated with the activation of the cytokine system and resultant elevated serum cytokine levels, particularly interleukin (IL)-6.[2] Activation of cytokines may also be secondary to trauma, including postsurgery. Alternatively, cytokine activation, particularly of the proinflammatory variety, may be a major contributory factor in autoimmune diseases, such as rheumatoid arthritis (RA).[3]

The concurrence of depressed T_3 production and cytokine activation in certain diseases and after trauma has led to suggestions that cytokine activation may be a major factor contributing to the thyroid hormone changes. In a study[4] of patients undergoing elective surgery, we showed that the changes in serum thyroid hormones preceded increased serum IL-6 concentrations and therefore concluded that the cytokine activation was unlikely to be the cause of the thyroid changes. However, other studies in a wide variety of NTIS patients have proposed the alternative conclusion based on the observed correlation of increased IL-6 and decreased T_3 concentrations.[5,6] The hypothesis that cytokines may contribute to inhibition of T_3 production from T_4 in NTIS therefore warranted further study.

RA is known to be associated with increased serum IL-6 concentrations with the degree of elevation being positively correlated with severity of disease.[7] Therefore, in the present study, we examined the interrelationships between plasma T_3 and serum IL-6 and related parameters in untreated and treated RA patients. Patients with noninflammatory musculoskeletal symptoms served as controls.

MATERIALS AND METHODS

Patients

The patient groups were as follows: group 1, 16 patients presenting for the first time as outpatients fulfilling the American Rheumatism Association (ARA) criteria for RA[8] who were previously untreated; group 2, 27 patients presenting for the first time to the outpatient department with noninflammatory musculoskeletal symptoms, comprising osteoarthritis and regional pain syndromes (tennis elbow, rotator cuff dysfunction); group 3, 35 patients revisiting the outpatient department with the diagnosis of RA already on treatment with disease modifying antirheumatic drugs, nonsteroid antiinflammatory drugs, or simple analgesics (excluding gold-containing medications and steroids); and group 4, 27 patients revisiting the outpatient department with noninflammatory musculoskeletal symptoms already on treatment with nonsteroidal antiinflammatory drugs and simple analgesics.

Males and females of any age and of any severity of disease were included in all groups. Treatment for other diseases did not disqualify, except patients treated with corticosteroids, amiodarone, or gold-containing drugs were excluded. Patients on treatment for thyroid dysfunction were not excluded, providing thyroid function tests were normal. Patients with untreated thyroid dysfunction were excluded.

All patients gave written informed consent and agreed to complete questionnaires relating to disease activity and dietary intake. The project plan was examined and approved by The Queen Elizabeth Hospital Ethics of Human Research Committee.

From the Departments of Clinical Chemistry and Rheumatology, The Queen Elizabeth Hospital Campus, North Western Adelaide Health Service, Woodville South, South Australia.

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Address reprint requests to M.L. Wellby, MD, Department of Clinical Chemistry, The Queen Elizabeth Hospital Campus, North Western Adelaide Health Service, Woodville Rd, Woodville South, South Australia 5011.

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Sampling and Laboratory Methods

Venepuncture blood samples were collected into glass tubes on ice for IL-6 estimation and into standard serum tubes for the other assays. The serum was separated and stored at −80°C until assay. Free T_4 (FT_4), freeT_3 (FT_3), and TSH were measured by chemiluminescence (ACS 180, Chiron Diagnostics, MA). Lower limit of detection was 0.011 μIU/L for TSH, 0.8 pmol/L for FT3, and 1.3 pmol/L for FT4. Intraassay coefficient of variation (CV) and interassay CVs were 3.6% to 4.7% and 4.9% to 6.3% (TSH), 1.4% to 3.8% and 4.2% to 6.2% (FT_3), and 2.8% to 5.5% and 3.2% to 6.6% (FT_4). Normal ranges were 0.4 to 4.5 μIU/L for TSH, 2.5 to 5.3 pmol/L for FT_3, and 11 to 26 pmol/L for FT_4. IL-6 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) ("Biotrak"; Amersham International, Buckinghamshire, UK) with a lower limit of detection of 0.1 pg/mL and CV of 7.0% (intraassay), 9% (interassay), and normal range of 0 to 14.9 pg/mL. C-reactive protein (CRP), an objective although nonspecific marker of inflammation, was measured by nephelometry (BNS, Behring, Marburg, Germany) using the latex-CRP method with a lower limit of detection of 0.175 mg/L, an intraassay CV of 2.3% to 4.4%, an interassay CV of 3.6% to 4.4%, and a normal range for CRP of less than 10 mg/L.

Dietary Questionnaires

As subnormal food intake, particularly of carbohydrates, leads to impaired peripheral production of T_3, each patient completed a comprehensive questionnaire designed to assess overall food intake and the pattern of carbohydrate, fat, and protein intake. The average normal diet was divided into 12 commonly eaten food groups. Patients were asked to list the estimated quantity eaten daily of each food group in the questionnaire. From this, a score from 0 to 5 for each food group was given by the same assessor who had no knowledge of the patient's group.

Statistical Analyses

Data were analyzed by the Kruskal-Wallis nonparametric analysis of variance (ANOVA) followed by multiple comparisons between groups using Dunn's test. Correlations between thyroid indices and IL-6 were tested using Spearman rank correlation tests. A critical value of $P < .05$ was adopted.

RESULTS

Age and Nutritional Index

No significant differences were found between the groups for either age or nutritional status, the mean values ± SEM being respectively from groups 1 to 4: age (years) 60.6 ± 4.4, 62.9 ± 3.3, 64.2 ± 2.1, and 65.7 ± 2.5; and nutritional index: 34.7 ± 3.6, 32.1 ± 1.9, 35.8 ± 1.2, and 32.6 ± 1.6. In addition, the relative contribution of carbohydrate, fat, and protein was similar in all groups.

Sex distribution of patients was similar in untreated RA and patients with noninflammatory musculoskeletal symptoms being respectively 44% male/56% female and 48% male/52% female. Treated RA patients consisted of 34% males and 66% females, while treated patients with noninflammatory symptoms consisted of 22% males and 78% females.

IL-6 and C-Reactive Protein

Serum IL-6 values in pg/mL (Fig 1A) in untreated RA patients (group 1) were significantly higher than those for patients with noninflammatory symptoms, either untreated (group 2; $P < .001$) or treated (group 4; $P < .001$). Treated RA patients (group 3) had significantly lower serum IL-6 values

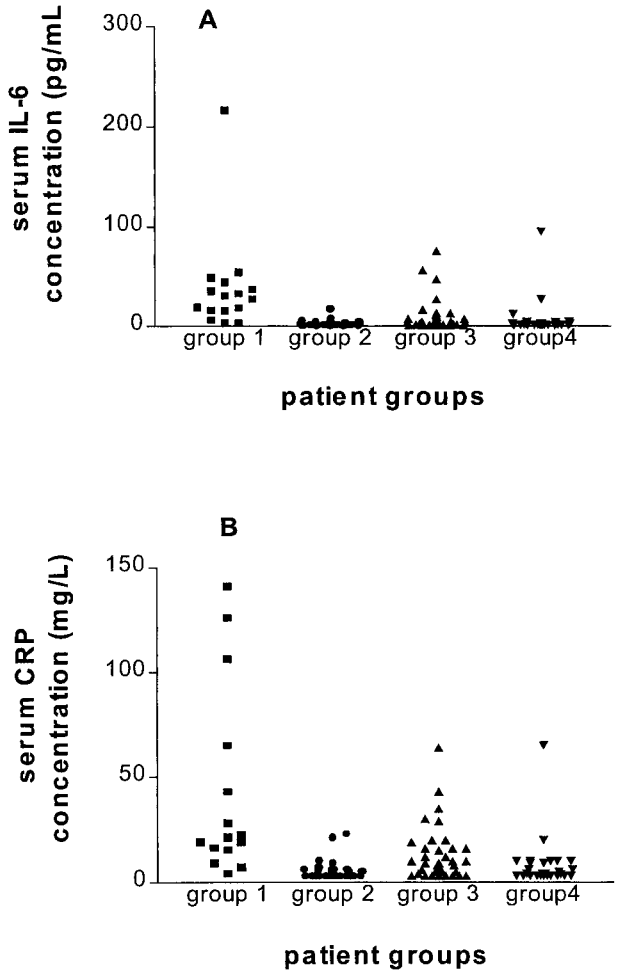

Fig 1. Serum (A) IL-6 and (B) C-reactive protein (CRP) concentrations in untreated RA patients (n = 16), group 1 (■); untreated patients with noninflammatory symptoms (n = 27), group 2(●); treated RA patients (n = 35), group 3(▲); and treated patients with noninflammatory symptoms (n = 27), group 4(▼). IL-6 concentrations were significantly elevated in group 1 patients compared with group 2 and group 4 patients ($P < .001$) and group 3 ($P < .01$); IL-6 concentrations of group 3 patients were significantly greater than those of group 2 ($P < .05$), Kruskal-Wallis ANOVA and Dunn's test. CRP concentrations were significantly greater in group 1 patients than group 2 and group 4 ($P < .001$) or group 3 ($P < .05$): CRP of group 3 patients was significantly greater than that of group 2 patients ($P < .05$), Kruskal-Wallis ANOVA, Dunn's test.

than untreated RA patients (group 1; $P < .01$), but were significantly higher than values in untreated patients with noninflammatory symptoms ($P < .05$). The serum CRP concentrations (Fig1B) displayed similar between group differences to serum IL-6 concentrations. Untreated RA patients had higher CRP concentrations than patients with noninflammatory symptoms ($P < .001$) and treated RA patients ($P < .05$). However, even with treatment, RA patients (group 3) had significantly elevated CRP concentrations compared with group 2 patients ($P < .05$).

Thyroid Tests

Notwithstanding the differences in IL-6 and CRP, no significant differences were found for either FT_3 or FT_4 between any

of the patient groups (Fig 2A and B, respectively). The mean values (± SEM) for serum FT_3 (pmol/L) were: group 1, 3.49 ± 0.15; group 2, 3.65 ± 0.15; group 3, 3.12 ± 0.09; and group 4, 3.32 ± 0.16. Serum TSH concentration was significantly higher in group 4 patients than in group 1 patients (Fig 2C; $P < .05$, Kruskal-Wallis ANOVA and Dunn's test). There were no significant differences between TSH values for the other patient groups.

Correlation Between Thyroid Indices and IL-6

Spearman rank correlations were performed between IL-6 and FT_3, FT_4, TSH, and CRP values in all groups of patients (Fig 3). No significant correlation was found between IL-6 and any of the thyroid indices in any patient group. A significant positive correlation ($r = .62$, $P < .0001$) was found for IL-6 and CRP only in group 3 (treated RA patients).

DISCUSSION

The most characteristic biochemical feature of NTIS is a subnormal serum T_3 concentration arising from depressed peripheral deiodination of T_4[1]. The primary aim of the present study was to examine the hypothesis that the depressed T_3 production arises, at least in part, from activation of the cytokine system and, in particular, from excessive IL-6 production.

To test this hypothesis, the disease of RA was chosen because of its chronicity and because of the major pathogenetic factor of synovial membrane IL-6 overproduction resulting in elevated serum IL-6 concentrations.[7,9] The pathogenetic significance of IL-6 in RA is supported by the reported correlation between disease activity and serum IL-6.[7,9] Serum IL-6 concentrations were elevated to 37.5 ± 12.5 pg/mL (mean ± SEM) in our untreated RA patients compared with the normal reference range of 0 to 14.9 pg/mL and compared with normal values in patients with noninflammatory musculoskeletal symptoms, namely a mean of 2.5 pg/mL. CRP levels in untreated RA patients were elevated compared with levels in patients with noninflammatory musculoskeletal symptoms and with levels in treated RA patients. This reflects the major systemic disturbance of RA. IL-6 and CRP levels positively correlated in treated RA, but not in untreated RA patients, presumably due to the much smaller number of patients, combined with a few very high levels of CRP in the latter group.

However, all serum thyroid levels were normal in our untreated RA patients. In particular, serum FT_3 concentrations ranged from 2.7 to 4.5 pmol/L, which is not significantly different from the normal range (2.5 to 5.3 pmol/L) or from the range of 2.5 to 6.0 pmol/L found in the patients with noninflammatory symptoms. Furthermore, no relationship was found between serum IL-6 and FT_3 values in the combined groups of untreated and treated RA patients. Hence, the present study does not substantiate the hypothesis that excess IL-6 activation is a primary cause of subnormal serum T_3 concentrations in NTIS or, at least not in patients with RA in which such cytokine activation has been well documented.[7]

Looking to other studies, significant decreases in plasma T_3 were observed when IL-6 was injected acutely into volunteers[10] or infused acutely into patients in doses to elevate serum levels to 1,418 pg/mL.[11] As the latter is a much higher IL-6 level than seen in our RA patients, it could be questioned whether the

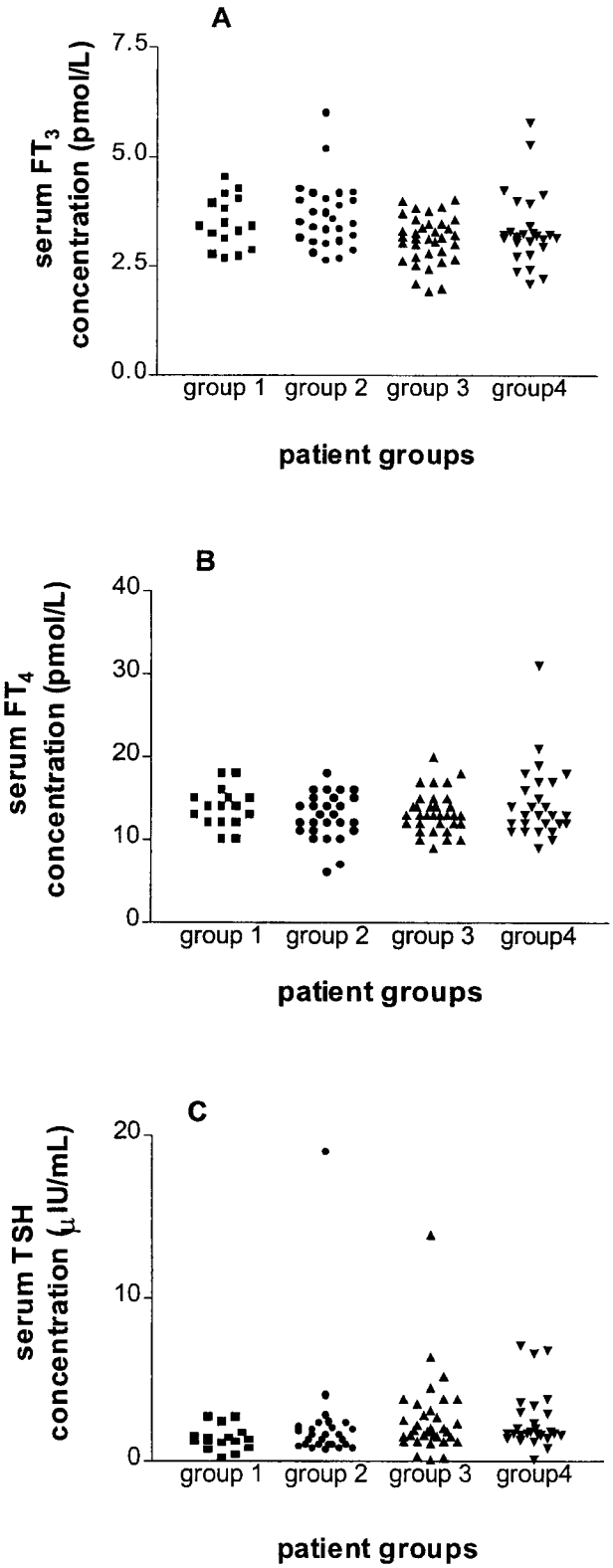

Fig 2. Serum concentrations of FT_3 (A), FT_4 (B), and TSH (C) in group 1 (■), 2 (●), 3 (▲), and 4 (▼) patients (n = 16, 27, 35, and 27, respectively). There were no significant differences between any of the patient groups with respect to either FT_3 or FT_4. TSH concentrations were significantly higher in group 4 than group 1 patients ($P < .05$) Kruskal-Wallis ANOVA, Dunn's test.

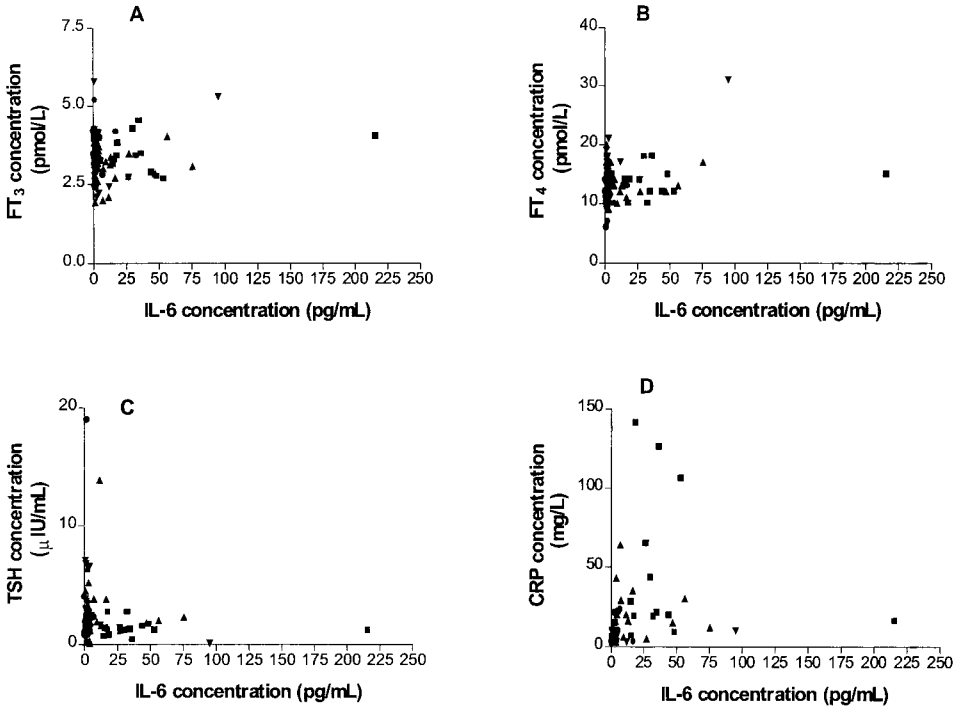

Fig 3. Relationship between serum IL-6 concentrations and (A) FT_3, (B) FT_4, (C) TSH, and (D) CRP concentrations for group 1(■), group 2 (●), group 3 (▲), and group 4 (▼) patients. No significant correlations were found for IL-6 with respect to FT_3, FT_4, or TSH for any of the patient groups, either individually or combined. IL-6 and CRP were positively correlated for group 3 ($r = .62$, $P < .0001$, n = 35) and the combined groups ($r = .60$, $P < .001$, n = 104), Spearman rank correlation.

degree of IL-6 elevation in our RA patients was sufficient to affect the peripheral deiodination of T_4. This question cannot be answered with certainty, as it is difficult to compare the effects of IL-6 elevations achieved pharmacologically with those seen as a result of a disease process in which the IL-6 elevation may be more effectual, particularly when the disease is chronic. Perhaps a more important question is whether IL-6 levels associated with subnormal serum T_3 in diseases other than RA are similar to the IL-6 levels found in our RA patients in whom T_3 is unchanged. Although it is difficult to make such comparisons because of different methods of measuring IL-6, there are studies, which show similar IL-6 elevations to those we found in RA are associated with subnormal serum T_3 levels.[5,6,12] Furthermore, these studies show an inverse relationship between serum IL-6 and T_3 in a variety of NTIS, both acute and chronic. However, Davies et al[6] reported that only intensive care patients had markedly decreased serum total T_3 and markedly elevated IL-6 levels, whereas those patients in the general hospital wards had slightly decreased total T_3, associated with IL-6 concentrations, which were similar to those found in our RA patients.

Accordingly, it seems that moderate chronic elevation of serum IL-6, as seen in untreated RA patients, is unlikely to be associated with measurable changes in serum T_3 concentration. The most likely situations in which elevated IL-6 and decreased serum T_3 coexist are when the IL-6 change is large and results from an acute process, such as in an intense illness or from injection of IL-6.

The relevance of the finding of inverse relationships between serum IL-6 and T_3 in NTIS patients[5,6,12] warrants further comment. Clearly, such a correlation does not necessarily imply a causal relationship. The elevated IL-6 may not be the cause, or at least not the only or the main cause of the decreased T_3. It is quite possible that associated stress or some other factor or factors are the cause of the T_3 changes. One study[6] showed inverse effects in a wide variety of illnesses, but not in renal diseases; the investigators concluding that factors other than IL-6, for example, stress, were leading to decreased T_3 production. This conclusion is consistent with results of our earlier study[4] in patients undergoing elective surgery in which we reported that FT_3 decreases preceded increases in IL-6. Clearly, IL-6 was not the cause of the T_3 changes under these conditions. Moreover, in the study in which an IL-6 infusion was administered, all subjects experienced generalized stress reactions as evidenced from significant body temperature increases.[11] Similarly, subcutaneous administration of IL-6 in normal volunteers was accompanied by a 3-fold increase in plasma cortisol, which could have been the major factor suppressing T_3 production from T_4[10].

On the other hand, a causal role for IL-6 in supression of T_3 is supported by the study of Boelen et al,[13] in which IL-6-knockout mice displayed smaller serum T_3 decreases in response to a number of induced "illnesses" (lipopolysaccharide, *Listeria monocytogenes*, or turpentine injection) than wild-type mice. These data suggest that IL-6 does contribute to the T_3 decrease. However, because there was a significant, albeit smaller, decrease in T_3 in the IL-6 knockout mice, there must be a major causative factor in addition to IL-6 leading to the T_3 decrease.

In any study of NTIS, the nutritional status of patients and animals should be considered in interpreting serum T_3 concentrations, as decreased energy intake, particularly of carbohydrate, will lead to rapid and marked decreases in serumT_3.[1] This factor has not been adequately addressed in many studies linking IL-6 and T_3 in disease states. However, this was not a factor in our study, because all patients were assessed by

dietary questionnaire, and the different patient groups showed no significant differences in nutritional index.

In conclusion, we have studied untreated RA patients as a model of NTIS and measured the relationship of serum thyroid hormone and IL-6 changes comparing these with measurements in patients with noninflammatory musculoskeletal symptoms. Although the expected increases in serum IL-6 were found in RA patients, serum FT_3 concentrations were quite normal. Accordingly, we have been unable to confirm in this model that a significant degree of IL-6 activation leads to the low T_3 state of NTIS. It is possible that the IL-6 increases in RA are insufficient to inhibit T_4 deiodination to T_3. However, other factors, such as stress, could be operative in producing the failure of deiodination of T_4 to T_3 in NTIS.

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